VIRGINIA:

IN THE CIRCUIT COURT OF FAIRFAX COUNTY

COMMONWEALTH OF VIRGINIA	)	
	)	
	)	Crim. No.: FE-2019-279
v.	)	
	)	
CLARK WATSON,	)	
	)	
Defendant	)	
	)	

MOTION FOR RECONSIDERATION AND
MOTION FOR AMENDED PROTECTIVE ORDER OF CYBERGENETICS

COMES NOW, Cybergenetics Corporation, by counsel, Brandon R. Shapiro, pursuant to §8.01-277 of the 1950 Code of Virginia, as amended, for the limited appearance to request this Honorable Court reconsider and amend the protective order issued requiring Cybergenetics to provide the TrueAllele source code for the following stated reasons:

1. Cybergenetics is a Pennsylvania corporation located at 160 North Craig Street, Suite 210, Pittsburgh, PA 15213. Cybergenetics is the owner of the TrueAllele® software, as well as its proprietary trade secret source code.

2. Cybergenetics licenses its TrueAllele technology to the Commonwealth's Department of Forensic Science (DFS) as an executable software program.

3. DFS has reported TrueAllele results as Certificates of Analysis (COA) in over eight hundred Virginia criminal cases.

4. Cybergenetics reported TrueAllele COAs in 2011 and 2012 in the Fairfax County child abduction case of *Commonwealth v. Jonathan Ramsey*. Cybergenetics Chief Scientist Dr. Mark Perlin testified in Fairfax Court in that case in June of 2012.

5. TrueAllele technology was found to be reliable in a December 17, 2013 Virginia admissibility decision by Chief Judge W. Allan Sharrett of the Sixth Judicial Circuit (Exhibit 1). Judge Sharrett ruled after holding a five-day Spencer hearing for TrueAllele match results in the double homicide case of *Commonwealth of Virginia v. Matthew Brady* in Colonial Heights County.

6. DFS does not have or need TrueAllele source code. Rather, the crime lab tests and validates its TrueAllele software in accordance with established federal validation guidelines and standards. Therefore, TrueAllele source code is not in the possession of the Commonwealth.

7. Cybergenetics and DFS respond to subpoena duces tecum (SDT) requests made by defendants. A prosecutor generally files a Motion to Quash (MTQ) on a SDT request for source code trade secrets. One such MTQ filed in 2013 in the Loudoun County case of *Commonwealth v. Darwin Bowman* is provided as an example (Exhibit 2). Courts grant these MTQs.

8. The Commonwealth's DFS produced a COA report that is part of the Commonwealth's evidence in this matter (Exhibit 3).

Certificate for an Out-of-state Subpoena

9. According to the docket in this matter, a certificate for an out-of-state subpoena was entered on August 23, 2019, directed to Cybergenetics. However, Cybergenetics never received service of a subpoena in this matter. Instead, Cybergenetics was contacted by Fairfax County ACA Lauren Hahn regarding this case on September 13, 2019.

10. Prosecutor Hahn forwarded an unsigned defense motion for a certificate for a subpoena to Cybergenetics via email. Cybergenetics voluntarily responded to the prosecutor's emergency request a few days later, on September 17, 2019, providing available materials.

11. No motion to compel, the subpoena, or motion affecting the scope of the subpoena was ever filed or served upon Cybergenetics.

12. Instead, a hearing for a motion to exclude DNA was held on August 14, 2020.

13. Cybergenetics was not represented at that hearing, and it had no opportunity to respond to the assertions against it, which allegations apparently resulted in the issuance of the August 25, 2020 order compelling production from Cybergenetics.

The Role of TrueAllele in DNA Analysis

14. TrueAllele is a probabilistic genotyping computer system that interprets DNA evidence using a statistical model. TrueAllele is used to analyze DNA evidence, particularly in cases where human review might be less reliable or not possible. (Exhibit 4)

15. Cybergenetics began developing TrueAllele 25 years ago, adding a mixture module 20 years ago. The casework system underwent many rounds of testing and model refinement over 10 years before it was used in criminal casework, with the current version 25 released in 2009.

16. The TrueAllele computer objectively infers genotypes from DNA data through statistical modeling, without reference to a known comparison genotype. In this way, TrueAllele computing avoids human examination bias, and provides a fair match statistic.

TrueAllele's Widespread Acceptance

17. TrueAllele has been used by Cybergenetics in over 900 criminal cases, with expert witness testimony given in over 90 trials. TrueAllele results have been reported in 44 of the 50 states. (Exhibit 4)

18. TrueAllele was used to identify human remains in the World Trade Center disaster, comparing 18,000 victim remains with 2,700 missing people.

19. Both prosecutors and defenders use TrueAllele for determining DNA match statistics. TrueAllele is also used by innocence projects and for post-conviction relief (*Connecticut v. Ralph Birch, Connecticut v. Shawn Henning, Georgia v. Johnny Lee Gates, Georgia v. Jimmy Meders, Georgia v. Kerry Robinson, Idaho v. Christopher Tapp, Indiana v. Roosevelt Glenn, Indiana v. Darryl Pinkins, Maryland v. William Jamison, Montana v. Paul Jenkins, Montana v. Freddie Lawrence, New Mexico v. Gregory Hobbs, Texas v. Lydell Grant, Washington v. Raymond Ben*).

20. TrueAllele's reliability has been confirmed in appellate precedent in Nebraska, New York, and Pennsylvania. *See: State of Nebraska v. Charles Simmer,* 302 Neb. 369 (2019); *People of New York v. John Wakefield*, N.Y. App. Div. LEXIS 6153, A-812-29 (2019); and *Commonwealth of Pennsylvania v. Kevin Foley*, 47 A.3d 882 (Pa. Super. 2012).

21. Cybergenetics agrees with the conclusions that were reached in the *Foley* case (Exhibit 5), which found that (i) scientists can validate the reliability of a computerized process even if the source code is not available to the public; (ii) it would not be possible to market TrueAllele if it were available for free; (iii) TrueAllele has been tested and validated.

TrueAllele is Considered to be Reliable

22. There is no genuine controversy as to the validity and reliability of the TrueAllele method. To the contrary, computer analysis of uncertain data using probability modeling is the scientific norm. Forensic science researchers see this as the best approach. (Exhibit 4)

23. Cybergenetics thoroughly tests its software before it is released.

24. Over thirty-five validation studies have been conducted by Cybergenetics and other groups to establish the reliability of the TrueAllele method and software. Eight of these

studies have been published in peer-reviewed scientific journals, for both laboratory-generated and casework DNA samples. Source code was not needed or used in any of these studies.

25. In the "peer-review" process, scientists describe their research methods, results and conclusions in a scientific paper, which they submit to a journal for publication. An editor at the journal has (at least) two independent and anonymous scientists in the field read the paper, assess its merits, and advise on the suitability of the manuscript for publication. The paper is then accepted, rejected, or sent back to the authors for revision and another round of review.

26. A "laboratory-generated" validation study uses data that has been synthesized in a DNA laboratory and is of known genotype composition. Five published peer reviewed TrueAllele papers of this type are: Perlin MW, Sinelnikov A. An information gap in DNA evidence interpretation. *PLoS ONE*. 2009;4(12):e8327; Ballantyne J, Hanson EK, Perlin MW. DNA mixture genotyping by probabilistic computer interpretation of binomially sampled laser captured cell populations: combining quantitative data for greater identification information. *Science & Justice*. 2013;52(2):103-14; Perlin MW, Hornyak J, Sugimoto G, Miller K. TrueAllele® genotype identification on DNA mixtures containing up to five unknown contributors. *Journal of Forensic Sciences*. 2015;60(4):857-868; Greenspoon SA, Schiermeier-Wood L, and Jenkins BC. Establishing the limits of TrueAllele® Casework: a validation study. *Journal of Forensic Sciences*. 2015;60(5):1263-1276; Bauer DW, Butt N, Hornyak JM, Perlin MW. Validating TrueAllele® interpretation of DNA mixtures containing up to ten unknown contributors. *Journal of Forensic Sciences*. 65(2):380-398, 2020.

27. The published peer-reviewed Greenspoon, Schiermeier-Wood, and Jenkins laboratory validation study listed above (Exhibit 6) was conducted by the Virginia DFS. Study author Lisa Schiermeier-Wood is the DFS analyst in the instant case.

28. A "casework" validation study uses DNA data exhibiting real-world issues developed by a crime laboratory in the course of their usual casework activity. Three published peer reviewed TrueAllele papers of this type are: Perlin MW, Legler MM, Spencer CE, Smith JL, Allan WP, Belrose JL, Duceman BW. Validating TrueAllele® DNA mixture interpretation. *Journal of Forensic Sciences*. 2011;56(6):1430-1447; Perlin MW, Belrose JL, Duceman BW. New York State TrueAllele® Casework validation study. *Journal of Forensic Sciences*. 2013;58(6):1458-66; Perlin MW, Dormer K, Hornyak J, Schiermeier-Wood L, and Greenspoon S, TrueAllele® Casework on Virginia DNA mixture evidence: computer and manual interpretation in 72 reported criminal cases. *PLoS ONE*. 2014:9(3):e92837.

29. The published peer-reviewed Perlin, Dormer, Hornyak, Schiermeier-Wood, and Greenspoon casework validation study listed above (Exhibit 7) was conducted jointly by Cybergenetics and the Virginia DFS. Study author Lisa Schiermeier-Wood is the DFS analyst in the instant case.

30. The Virginia DFS published a peer-reviewed article together with Virginia Commonwealth University showing how TrueAllele can be used as an information measuring system for DNA mixture data (Exhibit 8): Stokes NA, Stanciu CE, Brocato ER, Ehrhardt CJ, Greenspoon SA. Simplification of complex DNA profiles using front end cell separation T and probabilistic modeling. *Forensic Science International: Genetics*. 2018;36:205-12.

31. Conducting such validations is consistent with national forensic science standards for the validation of probabilistic genotyping methods.

32. Relevant Federal Bureau of Investigation (FBI) standards include (a) the 2010 Scientific Working Group on DNA Analysis Methods (SWGDAM) interpretation guidelines, (b)

the SWGDAM 2015 guidelines on probabilistic genotyping validation, and (c) the FBI Quality Assurance Standard (QAS) 2020 on DNA quality assurance validation.

33. Relevant American National Standards Institute (ANSI) and AAFS Standards Board (ASB) joint standards include (a) the 2018 Standard 020 for mixture validation and interpretation, (b) the 2019 Standard 040 for DNA interpretation and comparison, and (c) the 2020 Standard 018 for probabilistic genotyping validation.

34. Cybergenetics has documented how TrueAllele complies with all six of these national validation standards. These accepted scientific standards are based on empirical testing of probabilistic genotyping software on actual DNA data. Source code is not involved.

35. Regulatory bodies in New York and Virginia have had independent scientists review validation studies before they granted approval for their state crime laboratories to use TrueAllele for casework. The Virginia Scientific Advisory Committee and Forensic Science Board approved TrueAllele for use at DFS in 2013 (Exhibits 9 and 10).

36. TrueAllele has been admitted into evidence after opposition challenge in twenty-nine courts, located in California, Florida, Georgia, Indiana, Louisiana, Massachusetts, Nebraska, New York, Ohio, Pennsylvania, South Carolina, Tennessee, Virginia, Washington, United States, Northern Ireland and Australia.

37. Twenty-seven admissibility decisions in the United States are: People of California v. Dupree Langston, Kern County (Kelly-Frye), BF139247B, January 10, 2013; State of Florida v. Lajayvian Daniels, Palm Beach County (Frye), 2015CF009320AMB, October 31, 2018; State of Georgia v. Adedoja Bah, Douglas Judicial Circuit (Harper), 17CR00938, October 16, 2019; State of Georgia v. Alexander Battle, Ben Hill County (Harper), 16-CR-082, May 22, 2019; State of Georgia v. Monte Baugh and Thaddeus Howell, Coweta County (Harper), 2017 R

618, March 11, 2019; State of Georgia v. Nathaniel Day, Tifton Judicial Circuit (Harper), 2018CR141, October 23, 2019; State of Georgia v. Thaddus Nundra, South Georgia Circuit (Harper), 18-CR-134, January 21, 2019; State of Georgia v. Guy Sewell, Floyd County (Harper), 17-CR-1675 JFL004, August 7, 2019; State of Indiana v. Randal Coalter, Perry County (Daubert), 62C01-1703-MR-192, August 2, 2017; State of Indiana v. Dugniqio Forest, Vanderburgh County (Daubert), 82D03-1501-F2-566, June 3, 2016; State of Indiana v. Vaylen Glazebrook, Monroe County (Daubert), 53C02-1411-F1-1066, February 16, 2018; State of Indiana v. Malcolm Wade, Monroe County (Daubert), 53C02-1411-F3-1042, August 3, 2016; State of Louisiana v. Chattley Chesterfield and Samuel Nicolas, East Baton Rouge Parish (Daubert), 01-13-0316 (II), November 6, 2014; State of Louisiana v. Harold Houston, Jefferson Parish (Daubert), 16-3682, May 19, 2017; State of Louisiana v. Kyle Russ, East Baton Rouge Parish (Daubert), 01-14-0566, April 30, 2019; Commonwealth of Massachusetts v. Heidi Bartlett, Plymouth County (Daubert), PLCR2012-00157, May 25, 2016; State of Nebraska v. Charles Simmer, Douglas County (Daubert), CR16-1634, February 2, 2018; People of New York v. John Wakefield, Schenectady County (Frye), A-812-29, February 11, 2015; People of New York v. Casey Wilson, Chemung County (Frye), 2013-331, May 1, 2019; State of Ohio v. David Mathis, Cuyahoga County (Daubert), CR-16-611539-A, April 13, 2018; State of Ohio v. Maurice Shaw, Cuyahoga County (Daubert), CR-13-575691, October 10, 2014; Commonwealth of Pennsylvania v. Kevin Foley, Indiana County (Frye); State of South Carolina v. Jaquard Aiken, Beaufort County (Jones), 20121212-683, October 27, 2015; State of Tennessee v. Demontez Watkins, Davidson County (Daubert), 2017-C-1811, December 17, 2018; Commonwealth of Virginia v. Matthew Brady, Colonial Heights County (Spencer-Frye), CR11000494, July 26, 2013; State of Washington v. Emanuel Fair, King County (Frye), 10-1-

09274-5 SEA, January 12, 2017; United States v. Lenard Gibbs, Northern District of Georgia (Daubert), 1:17-CR-207, May 30, 2019.

38. The Pennsylvania Superior Court affirmed the Foley decision on February 15, 2012, 2012 PA Super 31, No. 2039 WDA 2009 (Exhibit 1). The New York State Supreme Court affirmed the Wilson decision on August 15, 2019, 175 A.D.3d 158 (3d Dep't 2019). The Nebraska Supreme Court affirmed the Simmer decision on November 1, 2019, 302 Neb. 369.

39. Chief Judge W. Allan Sharrett of the Sixth Judicial Circuit of Virginia found TrueAllele to be reliable in 2013 under the *Spencer* standard in Commonwealth of Virginia v. Matthew Brady (Exhibit 1).

40. The DFS TrueAllele COA (Exhibit 3) in this case reports that a match between the left chest area of Washim Khan's shirt and Clark Watson is minimally 180 quadrillion (1.80×10^{17}) times more probable than a coincidental match to an unrelated person. This likelihood ratio (LR) of 180 quadrillion provides an upper bound on the *false positive error rate*. Based on LR mathematics, the chance that someone who didn't contribute their DNA to the mixture has a match statistic as large as the reported LR is no more than one in 180 quadrillion.

Background on Software Source Code

41. People write a computer program in a programming language using "source code". This source code is later translated into computer-readable "executable" software. (Exhibit 4)

42. The source code details step-by-step human-readable instructions that describe to the computer and programmers how the program operates.

43. TrueAllele is written in MATLAB (for MATrix LABoratory), a high-level mathematical language for programming and visualizing numerical algorithms made by the

MathWorks (Natick, MA). Thus, source code is written in language that humans are capable of understanding, but only if they are fluent in reading, writing and interpreting the particular language that the program is written in.

44. TrueAllele has about 170,000 lines of computer source code, written by multiple programmers over two decades. The computer code is dense mathematical text. It can take hours for a person to read through even a few dozen lines of MATLAB to decipher what it does. Reading at ten lines per hour would entail eight and a half person-years to review all the source code.

45. It is wholly unrealistic to expect that reading through TrueAllele source code would yield meaningful information.

Why TrueAllele is a Trade Secret

46. People can easily copy a computer program if they have its source code. Source code contains the software design, engineering know-how, and algorithmic implementation of the entire computer program. (Exhibit 4)

47. Cybergenetics has invested millions of dollars over two decades to develop its TrueAllele system, the company's flagship product. Although the technology is patented, the source code itself is not disclosed by any patent and cannot be derived from any publicly disclosed source. Patent protection is not automatic, and litigation can cost tens of millions of dollars.

48. Cybergenetics considers the TrueAllele source code to be a trade secret. Cybergenetics does not disclose the source code to anyone outside the company. In fact, the source code has never been disclosed. The source code is not distributed to most employees of

Cybergenetics, and copies are not provided to individuals, businesses or government agencies that use or license the software.

49. The fact that the source code is kept secret provides Cybergenetics with a significant advantage over others who do not have access to the source code and do not have the programming know-how or are not willing to make the investment necessary to develop comparable software.

50. Cybergenetics operates in a highly competitive commercial environment. In recent years, at least ten other groups have developed similar software.

51. There is keen interest from competitors to find out how to replicate TrueAllele. The TrueAllele software represents a technological breakthrough that has not been successfully replicated by any other company as of this date.

52. Disclosure of the TrueAllele source code trade secret would cause irreparable harm to the company, enabling competitors to easily copy the company's proprietary products and services.

53. Ownership of the TrueAllele program and source code provides Cybergenetics with an advantage over its competitors who do not know the proprietary code and could not legally duplicate it.

54. Cybergenetics takes reasonable measures to protect the secrecy of the source code. For example, all information relating to the source code is housed on secure computers.

TrueAllele Source Code is a Trade Secret Protected by Virginia Law

55. TrueAllele source code is a trade secret that the law protects from disclosure.

a) It is well established that the law affords the owner of a trade secret protection "against the disclosure or unauthorized use of the trade secret." *MicroStrategy*

Inc. v. Li, 601 S.E.2d 580, 588 (Va. 2004) citing *Kewanee Oil Co. v. Bicron Corp.*, 416 U.S. 470, 475, (1974).

b) The Virginia Code defines a trade secret as information that (1) derives independent economic value from not being generally known and (2) is the subject of reasonable efforts to maintain its secrecy. Va.Code § 59.1–336, *Strategic Enter. Sols., Inc. v. Ikuma*, 77 Va. Cir. 179, 2008 WL 8201356 (Va. Cir. Ct. 2008).

c) Virginia Courts have recognized that software components, as part of a computer program, may constitute trade secrets that are afforded protection from disclosure. *MicroStrategy Inc.*, 601 S.E.2d at 588.

d) Cybergenetics' TrueAllele DNA interpretation software clearly satisfies the two components of the definition of a trade secret, and should therefore, be afforded protection from disclosure.

Irremediable Risks of Source Code Disclosure

56. Third party review of source code can divulge proprietary trade secrets wholly unrelated to reliability, but valuable to competitors. Once a review results in a release of hard-earned engineering know-how, that disclosure cannot be reversed. The source code reviewer's knowledge can be written into other software systems, shared with interested parties, or sold for profit. There are no adequate remedies for redress once this proprietary information has been released. (Exhibit 4)

57. The credibility and trustworthiness of retained witnesses who testify about the alleged need for forensic source code has been undermined in cross-examination (*New York v. Jaquan Collins, Pennsylvania v. Michael Robinson*). Permitting such individuals to see

proprietary information that is immaterial to a case is not reasonable, nor is it in the interest of justice.

58. Protective orders for source code are sometimes used in expensive civil litigation for patent infringement, which is not germane to criminal proceedings. Protective orders may fail to protect valuable trade secrets, leading to unwanted disclosure of proprietary designs, methods, and know-how (*Superspeed LLC v. Google*, United States District Court for the Southern District of Texas; *Bradford Technologies, Inc. v. NCV Software.com*, United States District Court for the Northern District of California; *Apple v. Samsung*, United States District Court for the Northern District of California; *Eli Lilly & Co. v. Gottstein*, United States Court of Appeals for the Second Circuit; *Smith & Fuller, PA v. Cooper Tire & Rubber Co.*, United States Court of Appeals for the Fifth Circuit).

59. There is no real effective remedy once a protective order is violated. Courts typically merely reimburse the fees that were incurred by the party whose secrets were revealed. In a case involving source code that is a trade secret, however, once the source code has been revealed in breach of a protective order, it generally loses its status as a trade secret. The genie can't be put back in the bottle, and reimbursement of legal fees does nothing to compensate for the loss of commercial value.

60. Cybergenetics uniquely provides accurate, objective, and neutral DNA identification information for criminal justice. TrueAllele DNA match results are used by both prosecution and defense for an unbiased statistical assessment of biological evidence. Crime laboratories rely on their validated TrueAllele systems for effective interpretation of complex DNA data. Jeopardizing the existence of Cybergenetics through a disclosure of its source code is unreasonable, and does not serve the interests of justice.

Why TrueAllele Source Code is Not Needed

61. Cybergenetics offers the TrueAllele software for license by crime labs and to other interested parties. (Exhibit 4)

62. Cybergenetics provides opposing experts the opportunity to review the TrueAllele process, examine results, and ask questions. This review can be done in Cybergenetics's Pittsburgh office, or through an Internet Skype-like meeting. Cybergenetics regularly explains the system, and the results obtained in a case, to both prosecution and defense. This introduction to the TrueAllele method, the case data, and the application of the method to the data, is a logical first step in understanding how the system works. Source code is not necessary.

63. The TrueAllele method is inherently objective, since the computer determines evidence genotypes without any knowledge of the comparison reference genotypes or user manipulation of DNA data. Hence there is no possibility of examination bias when determining genotypes from the DNA data. Match statistics, whether inclusionary or exclusionary, are calculated only afterwards by comparing evidence genotypes with reference genotypes. Source code is not needed to understand that the TrueAllele process is objective.

64. Cybergenetics provided discovery material for this case on an optical disc. The DVD contains documents related to TrueAllele's reliability, such as background reading, over thirty validation studies and publications, regulatory approvals, general acceptance, and admissibility rulings. There are tutorial videos that describe TrueAllele methods and explain how the system works, as well as continuing legal education talks. The VUIer™ software for reviewing TrueAllele results is provided (with both Windows and Macintosh installers), along with instructions and user manuals. Case-specific files were disclosed by the Virginia DFS.

Source code is not needed to access these materials, read the files, use the executable VUIer software, or examine the computer results.

65. TrueAllele processing is available on-line through Cloud computing. Therefore, the system's capability can be operated as an Internet service, without purchasing a product. Any party can operate TrueAllele on the Cloud, and process their own DNA case or validation data.

66. Moreover, Cybergenetics makes this TrueAllele Cloud capability available to opposing parties at no charge so that they can conduct their own testing (Exhibit 11). Source code is not needed for assessing TrueAllele reliability, which is done by testing the executable program on actual data.

67. Although the source code for TrueAllele is a secret, the methodology it employs and implements has been disclosed. Cybergenetics has published the core mathematics of TrueAllele's underlying mathematical model for over 20 years. These publications include peer-reviewed scientific papers (1995, 2001, 2009, and 2011), and patent specifications (2000 and 2001).

68. Cybergenetics provides a compilation of these mathematical methods in a single summary document (Exhibit 12). This information discloses TrueAllele's genotype modeling mechanism, and enables others to understand or replicate the basic method. Indeed, at least ten other groups have developed their own software that uses TrueAllele's linear mixture analysis approach. The source code is not necessary or helpful to understand or test the methodology or reliability of the analysis.

69. DFS scientist Dr. Susan Greenspoon is the principal scientist on Virginia validation studies for TrueAllele technology. Dr. Greenspoon has written, "The primary aims of validation work are to determine if the product performs as advertised, to develop an expertise in

the use of the product, to assess the accuracy, precision and reproducibility (where applicable) of the technology, and to understand its limitations. We have achieved these goals without the source code to TrueAllele® Casework, as is true for the many different technologies and products that we use daily in the laboratory." (Exhibit 13).

70. Moreover, as Dr. Greenspoon continued in her statement, "Our ability to use a given technology for forensic DNA profiling is verified by thorough validation work, not studying the source code. We have never requested the source code for the TrueAllele® Casework software because it was not necessary in order to determine the reliability of TrueAllele® Casework."

71. Access to source code does not help identify problems in commercial probabilistic genotyping software. In a peer-reviewed scientific paper (Exhibit 14), the developers of the commercial STRmix™ software wrote that "any miscode found that has been identified in STRmix™ development or use, was identified by examination of the program's output and not the source code. It would be nearly impossible to identify subtle errors in code by viewing the code. The identification has always been a result of comparison of the results produced by a program to some known control. The results of these comparisons then trigger the examination of a specific section of the code in order to discover the source of the discrepancy."

Courts Have Ruled that TrueAllele Source Code is Not Needed

72. Courts in Virginia and in other states have ruled that defendants do not need TrueAllele source code to assess the software's reliability.

73. In his 2013 *Spencer* decision on TrueAllele reliability, Chief Judge W. Allan Sharrett of the Sixth Judicial Circuit of Virginia denied a defendant's request for source code (Exhibit 1). He wrote, "The first [Daubert factor] is whether the science could and had been

tested. *Id.* at 593. Here, much is made of the inability to thoroughly test the TrueAllele protocol, because its source code is unknown. However, the Court places great emphasis on the observation in *Commonwealth* v. *Foley*, 38 A.3d 882, 2012 Pa. Super. 31 (Pa. Super. Ct 2012) that validation studies are the best tests of the reliability of source codes. In this case, validation studies have been performed with positive results. They have not shown that the TrueAllele system is junk science; they have shown, in fact, that it is reliable."

74. The Loudoun County Commonwealth's Attorney's Office was granted a 2013 Motion to Quash a Subpoena Duces Tecum in *Commonwealth of Virginia v. Darwin Bowman* (Exhibit 2). The MTQ noted: 'In regard to the first requested item, the Source Code or Pseudo code for TrueAllele is proprietary information. In fact, the Pennsylvania Superior Court, in a ruling upholding the validation of TrueAllele in Indiana County, Pa., addressed the defendant's claim that "no outside scientist can replicate or validate Dr. Perlin's methodology because his computer software is proprietary." *Commonwealth v. Foley*, 2012 PA Super 31, 38 A.3d 882, 888-89 (Pa. Super Ct., 2012). The Court went on to state that "Foley's third reason for exclusion is misleading because scientists can validate the reliability of a computerized process even if the 'source code' underlying that process is not available to the public. TrueAllele is proprietary software; it would not be possible to market TrueAllele if it were available for free." Id. at 889.'

75. The Commonwealth of Pennsylvania Superior Court decided the TrueAllele source code trade secret issue in their 2012 appellate admissibility ruling in *Commonwealth v. Kevin Foley* (Exhibit 5). Their opinion stated, 'Foley's third reason for exclusion is misleading because scientists can validate the reliability of a computerized process even if the "source code" underlying that process is not available to the public. TrueAllele is proprietary software; it would not be possible to market TrueAllele if it were available for free.'

76. The Pennsylvania Superior Court opinion continued, 'Nevertheless, TrueAllele has been tested and validated in peer-reviewed studies. One study used laboratory-generated DNA samples and found that quantitative analysis performed by TrueAllele was much more sensitive than qualitative analysis such as that performed by the FBI. See Perlin & Sinelnikov, An Information Gap in DNA Evidence Interpretation, 4 *PLoS ONE* e8327, at 10 (2009), available at http://dx.doi.org/10.1371/journal.pone.0008327. A recent paper entitled "Validating TrueAllele® DNA Mixture Interpretation" used DNA samples from actual cases and reached similar results. See Perlin et al., Validating TrueAllele® DNA Mixture Interpretation, 56 *Journal of Forensic Sciences* 1430 (2011). The study "validated the TrueAllele genetic calculator for DNA mixture interpretation" and found that "[w]hen a victim reference was available, the computer was four and a half orders of magnitude more efficacious than human review." Both of these papers were published in peer-reviewed journals; thus, their contents were reviewed by other scholars in the field.'

77. In a 2015 unpublished Decision for a Writ of Mandate, the California Court of Appeals ruled on why TrueAllele source code was not needed in *People v. Martell Chubbs* (Exhibit 15). Judge Willhite wrote "Chubbs has received extensive information regarding TrueAllele's methodology and underlying assumptions, but he has not demonstrated how TrueAllele's source code is necessary to his ability to test the reliability of its results. We therefore conclude that Chubbs has not made a prima facie showing of the particularized need for TrueAllele's source code."

78. In a 2015 Decision and Order, Chief Administrative Judge (Outside New York City) Michael Coccoma ruled on why TrueAllele source code was not needed in *People of New York v. John Wakefield* (Exhibit 16). Judge Coccoma wrote, 'Simply put, the Defendant's

Crawford argument is misplaced. The source code is not a witness, it is not testimonial in nature, and it is not "a surrogate for accusatory in-court testimony."'

79. In a 2016 Memorandum Order, Allegheny County Judge Jill Rangos ruled on why TrueAllele source code was not needed in *Commonwealth of Pennsylvania v. Michael Robinson* (Exhibit 17). Judge Rangos wrote, "As the defense has argued that *Foley* is not controlling on the question of materiality of the source code, this Court held a two-day hearing and considered expert testimony and argument. After considering the testimony, this Court determined that the source code is not material to the defendant's ability to pursue a defense."

80. Judge Rangos continued, "An order requiring Cybergenetics to produce the source code would be unreasonable, as release would have the potential to cause great harm to Cybergenetics. Rather than comply, Dr. Perlin could decline to act as a Commonwealth expert, thereby seriously handicapping the Commonwealth's case."

81. In a 2017 Findings of Fact and Conclusions of Law on Defense Motion to Compel Cybergenetics' TrueAllele Casework Source Code, King County Judge Mariane Spearman ruled on why TrueAllele source code was not needed in *State of Washington v. Emanuel Fair* (Exhibit 18). In her ten-page Order, Judge Spearman wrote, "The Defense has not articulated with particularity what material information, if any, could be found by reviewing the source code. As several experts who work in the field of forensic DNA testing have testified, an examination of the source code is not necessary in order to determine the reliability of TrueAllele and validate it for casework."

82. Judge Spearman continued, "The Defense has failed to meet its burden to show that disclosure of the source code is material and reasonable. Based upon the factual findings set forth above, this Court is not persuaded that a review of the source code is necessary in order to

determine whether TrueAllele is reliable. The defense demand for the source code is not material or reasonable because the testimony in this case from both state and defense experts establishes that scientists can confirm the reliability of TrueAllele without access to the source code. This testimony is consistent with the holding of other courts that have addressed this same issue."

83. Judge Spearman concluded, "Further, the usefulness of disclosing the source code is outweighed by a substantial risk of financial harm to Cybergenetics. Scientists can confirm the reliability of Trueallele without access to the source code. Dr. Perlin and Cybergenetics have a legitimate interest in keeping the source code, a trade secret, confidential."

Virginia DFS Analysts are Qualified to Testify About TrueAllele Evidence

84. The four trained and certified DFS TrueAllele scientists have learned the software's underlying scientific principles, and regularly testify about the computer's results to juries. The DFS analyst in this case, Lisa Schiermeier-Wood, took Cybergenetics training courses in TrueAllele science and software, and in 2012 completed an advanced course in solving DNA mixture cases using the system (Exhibit 19).

85. Moreover, DFS analyst Schiermeier-Wood is coauthor of two peer-reviewed TrueAllele validation studies that scientifically establish the system's reliability. *See*: Perlin MW, Dormer K, Hornyak J, Schiermeier-Wood L, and Greenspoon S, TrueAllele® Casework on Virginia DNA mixture evidence: computer and manual interpretation in 72 reported criminal cases. *PLoS ONE*. 2014:9(3):e92837; and Greenspoon SA, Schiermeier-Wood L, and Jenkins BC. Establishing the limits of TrueAllele® Casework: a validation study. *Journal of Forensic Sciences*. 2015;60(5):1263-1276 (Exhibits 6 and 7).

Cybergenetics Response to Prosecutor's Emergency Discovery Request

86. Cybergenetics responded to prosecutor ACA Hahn's emergency discovery request made on Friday, September 13, 2019. The company was not acting to comply with any subpoena or court order; indeed, one did not even exist at the time. Rather, Cybergenetics voluntarily assisted the Commonwealth in its usual helpful manner. Within several business days, on Tuesday, September 17, 2019, the company responded to informal requests listed in a draft "Defendant's Motion for a Certificate to compel the production of documents" by providing 4 GB of available documents (Exhibit 20) and additional material.

87. Cybergenetics standard disclosure materials include an invitation to review TrueAllele source code under confidentiality. Following up on that offer, the defendant's attorney requested Cybergenetics standard non-disclosure agreement (NDA) for out-of-court confidential source code disclosure. On September 26, 2019, Cybergenetics voluntarily sent him a draft NDA. This NDA represented a negotiable out-of-court offer, and was not a response to any subpoena or court order (which did not exist at the time). Cybergenetics had told ACA Hahn that the company was very flexible, and was happy to remove cost requirements or location restrictions from the agreement. Evidently Cybergenetics' message about its NDA flexibility was not relayed to the defense.

Cybergenetics is Has Made its TrueAllele Technology Available to the Defendant

88. Cybergenetics believes in transparency. The TrueAllele algorithms have been published. Moreover, Cybergenetics has collated descriptions of its algorithms, and provided them to the defense.

89. Cybergenetics believes in transparency. Source code review is not needed for any scientific assessment of TrueAllele operation or reliability. Nonetheless, in light of the Protective Order, Cybergenetics has made TrueAllele source code available for inspection by the

defense team during normal business hours at the law offices of Carroll & Nuttall, located near the Courthouse at 10521 Judicial Drive, Suite 110, Fairfax, VA 22030.

90. Cybergenetics believes in transparency. Empirical testing of interpretation software on empirical DNA is the scientific and legal standard for assessing reliability. Toward that end, we again remind the defense of Cybergenetics' standing offer to have free access to the TrueAllele application software, so that the defense can test the system at no cost.

RESPECTFULLY SUBMITTED

Brandon R. Shapiro

CYBERGENETICS
By Counsel

CARROLL & NUTTALL, P.C.

Brandon R. Shapiro

Brandon R. Shapiro, Esquire
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Fairfax, Virginia 22030
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Facsimile: 703-273-7207
Email: brandon.shapiro@carrollnuttall.com
Counsel for Cybergenetics

CERTIFICATE OF SERVICE

I hereby certify that, on the 23rd day of September 2020, a true and correct copy of the foregoing was hand-delivered to the following:

Bryan Kennedy, Senior Assistant Public Defender
4103 Chain Bridge Road
Suite 500
Fairfax, VA 22030
bkennedy@fai.idc.virginia.gov

Lauren Hahn, Esq.
Assistant Commonwealth's Attorney
4110 Chain Bridge Road
Suite 114
Fairfax, VA 22030
lauren.hahn@fairfaxcounty.gov

Brandon R. Shapiro

EXHIBIT 1

VIRGINIA:

IN THE CIRCUIT COURT FOR THE CITY OF COLONIAL HEIGHTS

COMMONWEALTH OF VIRGINIA,

v. Case Nos. CR11-465-01,-02,-03 & -04
and CR11-494-01,-02,-03, & -04

MATTHEW FRANKLIN BRADY

ORDER FOR ADMISSIBILTY OF DNA EVIDENCE

CAME ON July 26, 2013, the parties to consider the hearing for the admissibility of the DNA evidence and certificate of analysis in the Matthew Franklin Brady case. The Commonwealth was represented by Warren Von Schuch, Senior Special Assistant Commonwealth and A. Gray Collins, III, Deputy Commonwealth Attorney, while the defendant was represented by Stephanie Miller, lead counsel from the Capital Defender's Office, Joseph Vigneri, Capital Defender, Jessica Bulos, Assistant Capital Defender, and Jon Thornbrugh, local counsel for the defendant. The defendant Matthew Franklin Brady was present during the five day hearing.

After reading the opening statements, hearing five days of testimony and hearing oral arguments, which is hereby incorporated by reference, the Court FINDS and states as follows:

The defense's motion to submit written argument is denied, as it would not assist the Court in the determination of the issues before it. The Court, therefore, is prepared, based upon the evidence it has heard and the arguments of counsel, to offer and to render its opinion.

Context is important: this is an admissibility hearing, not a hearing judging the weight of the evidence; and we have arrived at this point after a long process of legal development that

spans at least three quarters of a century.

The standard that the Court starts with is set forth in *Billips v. Commonwealth*, 274 Va. 805, 810, 652 S.E.2d 99, 102 (2007), where the Virginia Supreme Court stated that the burden of making a prima facie showing regarding the foundation of such evidence rests upon the proponent of the evidence. In this case, this means that the Commonwealth must initially make a prima facie case of the reliability of the scientific method offered.

The background for the admissibility of expert testimony dates back to the 1923 so-called "Frye test," wherein the Court stated that the trial court must be convinced not only of the reliability of the scientific evidence, but also of its general acceptance within the scientific community. *Frye v. United States*, 293 F. 1013 (D.C. Cir. 1923).

Over the years, *Frye* eroded. In *Ellis v. Int'l Playtex, Inc.*, 745 F.2d 292 (4th Cir. 1984), the Fourth Circuit noted that the *Frye* rule had come under increasing attack because of the importance it placed on the judge's subjective ability to "count heads" among experts in the scientific community. *Id.* at 304.

Critics and courts that have rejected *Frye* have argued that the acceptability of scientific data should be debated by experts in front of the jury in an era when scientific data is playing an increasingly important role at trial. *Id.* at 304. Thus, *Frye* began to erode because there was no reason not to let the jury see and evaluate the same data that experts were relying on to reach their conclusions.

In Virginia, in *Walrod v. Matthews*, 210 Va. 382, 388, 171 S.E.2d 180, 185 (1969), our Supreme Court observed that "[i]n matters of this kind which are not of common knowledge we must accept the opinion of experts . . . Evidence of this kind is competent, unless it is palpably absurd, and it is not made incompetent by the fact that other experts may have reached another conclusion. Always it should be scrutinized with care, but the manner in which it is weighed has nothing do with its

admissibility." *Id.* at 389, 171 S.E.2d at 185-86. The matter before this Court is not one of common knowledge.

Two decades later, the Virginia Supreme Court in *Spencer v. Commonwealth*, 240 Va. 78, 393 S.E.2d 609 (1990) put a finer point on *Walrod,* and elaborated that "[w]ide discretion must be vested in the trial court to determine, when unfamiliar scientific evidence is offered, whether the evidence is so inherently unreliable that a lay jury must be shielded from it, or whether it is of such character that the jury may safely be left to determine credibility for itself." *Id.* at 98, 393 S.E.2d at 621.

Thus, the Court reads together *Billips*, *Walrod*, and *Spencer*.

The Commonwealth must make a prima facie case of the reliability of the scientific method offered. In considering and asking itself what that prima facie case is, the Court does not engage in a *Frye* test. Rather, it starts with the proposition stated in *Walrod,* that evidence of this kind is competent, unless it is palpably absurd; and elaborated upon in *Spencer,* that the test is whether evidence is so inherently unreliable that a lay jury must be shielded from it.

Two principles emerge, each of them long-standing traditions in Virginia jurisprudence. One is the principle of judicial restraint, that yields to the trier of fact in determining matters, to the greatest extent possible; the second, a principle of trust in our triers of fact, and in particular, a principle of trust in our system of trial by jury.

In determining the *Billips* threshold of evaluating a prima facie case, the Court is guided, but not bound by, the factors in *Daubert v. Merrell Dow Pharmaceuticals*, 509 U.S. 579 (1993), to which case both Counsel have referred.

The Court observes the following in examining the four *Daubert* factors:

The first is whether the science could and had been tested. *Id.* at 593. Here, much is made of the inability to thoroughly test the TrueAllele protocol, because its source code is unknown.

However, the Court places great emphasis on the observation in *Commonwealth v. Foley*, 38 A.3d 882, 2012 Pa. Super. 31 (Pa. Super. Ct. 2012) that validation studies are the best tests of the reliability of source codes. In this case, validation studies have been performed with positive results. They have not shown that the TrueAllele system is junk science; they have shown, in fact, that it is reliable.

The second factor in *Daubert* is whether the protocol has been subjected to peer review and publication. *Daubert*, 509 U.S. at 593. There have been a number of peer-reviewed articles and peer-reviewed publications regarding TrueAllele. Indeed, the Division of Forensic Science has its own validation study that is, by its nature, a peer review of the TrueAllele system. In the Court's opinion, then, TrueAllele has been subjected to peer review and has had several peer-reviewed published studies.

The third factor in *Daubert* is the error rate and the standards of controlling the operation of the technique. *Id.* at 594. Certainly there are rates of error here, as might be expected with any scientific method of this sort; but there is no evidence that these rates of error are unacceptable, or compromise the validity of TrueAllele. The Division of Forensic Science clearly found them acceptable.

TrueAllele, additionally, has its own standards for controlling the operation of the technique. The Court notes the rigorous training with which Ms. Greenspoon and her staff at the Division of Forensic Science were provided, and the continuing support, as well. Further, TrueAllele utilizes widely accepted standards: MCMC, Bayesian theory, MATLAB and probabilistic modeling.

Finally, the fourth *Daubert* factor is the question of general acceptance, *Id.* at 594, though this factor is not controlling. There was, for example, no acceptance, much less general acceptance of the science approved in *Spencer* when it was decided.

However, general acceptance is a factor that is relevant in this case. The Court notes that TrueAllele has been accepted by NIST, and Dr. Perlin has conducted extensive lectures and conferences concerning it. The Court infers that he has done so for a number of years, and that he continues to do so.

It is also important to note that Virginia's Division of Forensic Science described TrueAllele as a valuable tool that has been held admissible in courts in Virginia, in other states in the United States, and in the United Kingdom. Additionally, Dr. Perlin has testified in five circuit courts in Virginia.

The fact that Dr. Perlin has previously been accepted as an expert in courts in Virginia, and that his testimony has been admissible is also of some importance, though neither controlling nor determinative, because its admissibility was not contested in those cases.

TrueAllele has also been accepted by New York State, and was used significantly in the September 11th bombing investigation. TrueAllele has certainly found greater acceptance than the analytical techniques utilized in *Spencer* enjoyed at the time of their acceptance.

In looking at the *Daubert* factors, then, and considering them in applying the law in *Walrod* and *Spencer*, the Court's opinion is that the Commonwealth has, pursuant to *Billips*, made a prima facie case of the reliability of the scientific method offered. The Court further finds that evidence offered under TrueAllele is not palpably absurd, and it is not so inherently unreliable that a lay jury must be shielded from it.

TrueAllele is , indeed, of such character that a jury may safely be left to consider all scientific evidence before it at the time of trial, and consistent with the instructions which will be given by the Court, the jury may be safely left to determine credibility for itself.

After reviewing the facts and analysis above, IT IS THEREFORE ADJUDGED, ORDERED AND DECREED the following:

1. The Commonwealth has made a prima facie case of the reliability of the TrueAllele scientific method offered.
2. The evidence offered under TrueAllele is not palpably absurd,
3. The evidence is not so inherently unreliable that a lay jury must be shielded from it.
4. The evidence is of such character that a jury may safely be left to consider all scientific evidence before it at the time of trial, and that consistent with the instructions, which will be given by the Court, the jury may be safely left to determine credibility for itself.

It is further ORDERED that the DNA evidence, including the certificate of analysis by the TrueAllele system, is hereby admissible at all further hearings and trials.

ENTERED: 12/17/13

JUDGE

We ask for this:

William Bray, Esquire
Commonwealth's Attorney for the City of Colonial Heights
550 Boulevard,
Colonial Heights, VA 23834
(804) 520-9293
(804) 520-9229 (fax)

A COPY, TESTE:
STACY L. STAFFORD, CLERK
COLONIAL HEIGHTS CIRCUIT COURT
BY: Deputy Clerk

William Davenport, Esquire
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SEEN AND OBJECTED TO:

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EXHIBIT 2

V I R G I N I A
IN THE CIRCUIT COURT FOR LOUDOUN COUNTY

COMMONWEALTH OF VIRGINIA	:	
	:	
v.	:	
	:	
DARWIN BOWMAN,	:	**CASE NO. 22005**
Defendant.	:	

MOTION TO QUASH SUBPOENA DUCES TECUM

COMES NOW Cybergenetics Corporation, by Counsel, and moves the Court to quash the subpoena duces tecum requested by the Defendant, and in support thereof states as follows:

1. The defendant, Darwin Bowman, is charged with several crimes, including Capital Murder, in violation of Virginia Code Section 18.2-31.
2. The Virginia Department of Forensic Science contracted with Cybergenetics Corporation to conduct an analysis of DNA recovered at the scene of the crime.
3. The defense has requested a subpoena duces tecum requiring Cybergenetics Corporation to provide several things including:
    - The source code or pseudo code for TrueAllele©;
    - An executable version of TrueAllele©;
    - Case-Specific data:
        - All specific input data and files for work done in conjunction with the Supplemental report of July 14, 2011, relating to FS Lab #1881 (Virginia Department of Forensic Sciences);
        - All specific output data and files for work done in conjunction with the Supplemental report of July 14, 2011, relating to FS Lab #1881 (Virginia Department of Forensic Sciences)'
        - The specific run parameters for the sample in this case.

- Validation studies:
    - The specific input data and files
    - All specific output data and files;
    - The specific run parameters for the validation samples to include:
        - Samples described in "Validating TrueAllele DNA Mixture Interpretation", Perlin *et al., J.Forensic Sci,* 2011, 56(6)
        - All other samples that comprise the basis for the accuracy and reliability of TrueAllele©, including both published and unpublished data.

4. Va. Sup. Ct. R. 3A:12 (b) states that "Where subpoenaed writings and objects are of such nature or content that disclosure to other parties would be unduly prejudicial, the court, upon written motion and notice to all parties, may grant such relief as it deems appropriate, including limiting disclosure, removal, and copying.."

5. In regard to the first requested item, the Source Code or Pseudo code for TrueAllele© is proprietary information. In fact, the Pennsylvania Superior Court, in a ruling upholding the validation of TrueAllele© in Indiana County, Pa., addressed the defendant's claim that "no outside scientist can replicate or validate Dr. Perlin's methodology because his computer software is proprietary." Commonwealth v. Foley, 2012 PA Super 31, 38 A.3d 882, 888-89 (Pa. Super Ct., 2012). The Court went on to state that "Foley's third reason for exclusion is misleading because scientists can validate the reliability of a computerized process even if the 'source code' underlying that process is not available to the public. TrueAllele is proprietary software; it would not be possible to market TrueAllele if it were available for free." Id. at 889.

6. The source code (or a pseudo code) is a trade secret of Cybergenetics Corporation.

Disclosure of this proprietary material would make it impossible for the company to provide the commercial technology.

7. Both of the defense experts in this case develop their own software and provide commercial services based on that software. As this essentially makes them competitors of Cybergenetics, the release of trade secrets to direct competitors would be unduly prejudicial to Dr. Perlin and Cybergentics Corporation. Further this Court, as a matter of public policy should not be placed in a position of unbalancing the scales between commercial competitors.
8. Dr. Perlin or a representative of Cybergenetics Corporation is willing to meet with the defense (either in person or via an internet meeting) both to go over the results of this case and to explain to them on a TrueAllele© computer how the system works.
9. In regards to the second item requested by the defense, the base price of a TrueAllele© system if $60,000, and is made available for purchase to government DNA laboratories. Cybergenetics does not provide free systems.
10. Dr. Perlin or a representative of Cybergenetics Corporation is willing to meet with the defense (either in person or through an internet meeting) both to go over the results of this case and to explain to them on a TrueAllele© computer how the system works.
11. In regards to the third item requested by the defense, the case specific data relating to the Supplemental Report of July 14, 2011 will be provided to the defense.
12. In regard to the fourth item requested by the defense, Cybergenetics corporation works with data files only, and has no access to the underlying biological samples.

13. The data files addressed in these validation studies are related to criminal cases in other jurisdictions. Cybergenetics Corporation does not feel it has the authority to release personal and confidential records.
14. Cybergenetics Corporation is willing to conduct additional TrueAllele© testing on a limited set of defense-provided data to further their understanding of the system, its operation, and its reliability.
15. The two experts for the defense, Dr. Rudin and Dr. Lohmeuller are acting as experts in a Frye hearing in Southern Virginia challenging TrueAllele© and it's software. These two experts, who are conducting essentially the same hearing less than two months after the scheduled date of their testimony in Loudoun County, have requested neither an executable copy of TrueAllele© nor the source code requested in this case. In fact no subpoena duces tecum was requested in that case for any items. It is baffling how the defense is able to prepare for the exact same hearing in another jurisdiction with the same experts without these items while the defense in this case finds them necessary.

For the above stated reasons, the Commonwealth respectfully requests that her motion be granted and the Court enter an order quashing items one (source code), two (executable version of TrueAllele©), and four (requesting data files in relation to the validation studies) of the above-mentioned *subpoenas duces tecum*.

Respectfully Submitted,
COMMONWEALTH OF VIRGINIA

Ryan W. Perry
Assistant Commonwealth's Attorney
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Office: (703) 777-0242
Facsimile: (703) 777-0160
oca@loudoun.gov

CERTIFICATE OF SERVICE

I, Ryan W. Perry, hereby certify that on this 24th day of May, 2013, a true copy of this Motion to Quash was electronically mailed to Jonathan Shapiro, counsel for the defendant.

Ryan W. Perry

EXHIBIT 3

Commonwealth of Virginia
DEPARTMENT OF FORENSIC SCIENCE

ORIGINAL

CERTIFICATE OF ANALYSIS

Central Laboratory
700 N. 5th Street
Richmond, VA 23219

Tel. No.: (804) 786-4707
Fax: (804) 786-6907

May 16, 2019

TO: SUSAN SHARP
SUSAN ANDERTON
FAIRFAX COUNTY POLICE DEPARTMENT
MCCONNELL PSTOC
4890 ALLIANCE DRIVE
FAIRFAX, VA 22030

SUPPLEMENTAL REPORT

FS Lab # N15-348

Your Case #: 20143340137

Victim(s): KHAN, Washim Bar

Suspect(s): WATSON, Clark Devell

Evidence Submitted By:	Susan Sharp	Date Received:	01/14/2015
Item 1	Buccal swabs from Washim Khan		
Item 2	Shirt from Washim Khan		
Evidence Submitted By:	Susan Anderton	Date Received:	02/15/2019
Item 10	Buccal swabs from Clark Watson		

STATISTICAL ANALYSIS METHODS

- The DNA PowerPlex® 16 and PowerPlex® Fusion profiles referenced in this report were previously developed and addressed in Certificates of Analysis dated January 8, 2016 and March 5, 2019.
- The TrueAllele® Casework system processed each evidence item in independent replicate computer analyses in which possible DNA contributor genotypes were inferred from the evidence profiles.
  - The term "genotypes" used in this context refers to a probability distribution over allele pairs.
- The likelihood ratio statistical method addressed below has been applied in accordance with the Scientific Working Group on DNA Analysis Methods (SWGDAM) 2017 Interpretation Guidelines and Departmental procedures.
- The DNA match statistics calculated herein used the population allele frequencies generated by the Virginia Department of Forensic Science, and a theta co-ancestry coefficient of 1%.
- The D12S391, DYS391 and Amelogenin loci are not used for statistical purposes.

RESULTS:

Item 1 - Buccal swabs from Washim Khan
Item 2 - Shirt from Washim Khan
Item 10 - Buccal swabs from Clark Watson

Assuming the DNA profile data previously developed from the left chest area of Washim Khan's shirt is a mixture of one or two unknown contributors and Washim Khan, TrueAllele® Casework system objectively inferred genotypes solely from these data. Two or three unknown contributors were also considered. Following duplicate/reproducible analyses, the computer then compared each inferred evidence contributor genotype to the provided reference genotype (Clark Watson), relative to reference populations, to compute likelihood ratio (LR) DNA match statistics.

Commonwealth of Virginia
DEPARTMENT OF FORENSIC SCIENCE

ORIGINAL

CERTIFICATE OF ANALYSIS

Fairfax County Police Department
FS Lab # N15-348 SUPPLEMENTAL REPORT
Your Case # 20143340137
May 16, 2019

Based on these results, Clark Watson cannot be eliminated as a contributor to this DNA mixture profile. A match between the left chest area of Washim Khan's shirt and Clark Watson is:

180 quadrillion times more probable than a coincidental match to an unrelated African American person,
6.3 quintillion times more probable than a coincidental match to an unrelated Caucasian person, and
6.5 quintillion times more probable than a coincidental match to an unrelated Hispanic person.

Date(s) of Testing: 3/22/19 - 5/16/19. Supporting examination documentation is maintained in the case file. The above listed methods are those approved for use at the time of analysis. Current methods can be found in the Forensic Biology Procedures Manuals, which can be found at www.dfs.virginia.gov/documentation-publications/manuals/.

The disposition of the evidence and the results of other requested examinations are the subject of another report.

Attest:

I certify that I performed the above analysis or examination as an employee of the Department of Forensic Science and that the above is an accurate record of the results and interpretations of that analysis or examination.

Lisa C. Schiermeier-Wood
Forensic Scientist

LCS

EXHIBIT 4

DECLARATION OF MARK W. PERLIN

I, Mark W. Perlin, declare I have personal knowledge of the following, and if called upon to do so, could and would testify competently to the matters contained herein:

1. I hold the following academic degrees: a B.A. in Chemistry from SUNY/ Binghamton, a Ph.D. in Mathematics from CUNY/Graduate School, an M.D. from the University of Chicago Pritzker School of Medicine, and a Ph.D. in Computer Science from Carnegie Mellon University. I have been issued thirteen patents. Prior to founding my own technology company, I was a senior research faculty member of Carnegie Mellon University's School of Computer Science. I have been qualified to testify as an expert in thirty-five jurisdictions. I am currently a scholar-in-residence faculty member in the Forensic Science and Law program at Duquesne University.

2. I reside at 5885 Marlborough Road, Pittsburgh, PA 15217.

3. Cybergenetics is a Pennsylvania corporation located at 160 North Craig Street, Suite 210, Pittsburgh, PA 15213. Cybergenetics is the owner of the TrueAllele software, as well as its proprietary source code.

The Role of TrueAllele in DNA Analysis

4. TrueAllele is a probabilistic genotyping computer system that interprets DNA evidence using a statistical model.

5. TrueAllele is used to analyze DNA evidence, particularly in cases where human review might be less reliable or not possible.

6. A definite genotype can be readily determined when abundant DNA from one person produces unambiguous genetic data.

7. However, when data signals are less definitive, or when two or more people contribute to the evidence, uncertainty arises.

8. This uncertainty is expressed in the derived contributor genotype, which may describe different genetic identity possibilities.

9. Such genotype uncertainty may translate into reduced identification information when a comparison is made with a suspect.

10. The DNA identification task can thus be understood as a two-step process:

(1.) objectively inferring genotypes from evidence data, accounting for allele pair uncertainty using probability, and

(2.) subsequently matching genotypes, comparing evidence with a suspect relative to a population, to express the strength of association using probability.

11. The match strength is reported as a single number, the likelihood ratio (LR), which quantifies the change in identification information produced by having examined the DNA evidence.

12. The TrueAllele® Casework system is Cybergenetics' computer implementation of this two-step DNA identification inference approach.

13. Cybergenetics began developing TrueAllele 25 years ago, adding a mixture module 20 years ago.

14. The casework system underwent many rounds of testing and model refinement over 10 years before it was used in criminal casework, with the current version 25 released in 2009.

15. The TrueAllele computer objectively infers genotypes from DNA data through statistical modeling, without reference to a known comparison genotype.

16. To preserve the identification information present in the data, the system represents genotype uncertainty using probability.

17. These probabilistic genotypes are stored on a relational database.

18. Subsequent comparison with suspects or other individuals provides identification information that can be used as evidence.

TrueAllele's Widespread Acceptance

19. TrueAllele has been used in over 900 criminal cases, with expert witness testimony given in over 90 trials. TrueAllele results have been reported in 44 of the 50 states.

20. Courts accepting TrueAllele evidence include California, Florida, Georgia, Idaho, Indiana, Louisiana, Maryland, Massachusetts, Michigan, Nebraska, New Hampshire, New York, Ohio, Pennsylvania, South Carolina, Tennessee, Texas, Virginia, Washington, West Virginia, Wyoming, United States (Northern District of Georgia, Middle and Eastern Districts of Louisiana, Eastern District of Virginia), United States Marine Corps, Northern Ireland, and Australia.

21. Over 10 crime laboratories have purchased the TrueAllele system for their own in-

house use, and 8 labs are on-line with their validated systems.

22. TrueAllele was used to identify human remains in the World Trade Center disaster, comparing 18,000 victim remains with 2,700 missing people.

23. Both prosecutors and defenders use TrueAllele for determining DNA match statistics. TrueAllele is also used by innocence projects and for post-conviction relief (*Connecticut v. Ralph Birch, Connecticut v. Shawn Henning, Georgia v. Johnny Lee Gates, Georgia v. Jimmy Meders, Georgia v. Kerry Robinson, Idaho v. Christopher Tapp, Indiana v. Roosevelt Glenn, Indiana v. Darryl Pinkins, Maryland v. William Jamison, Montana v. Paul Jenkins, Montana v. Freddie Lawrence, New Mexico v. Gregory Hobbs, Texas v. Lydell Grant, Washington v. Raymond Ben*).

24. TrueAllele's reliability has been confirmed in appellate precedent in Nebraska, New York, and Pennsylvania. *See: State of Nebraska v. Charles Simmer,* 302 Neb. 369 (2019); *People of New York v. John Wakefield*, N.Y. App. Div. LEXIS 6153, A-812-29 (2019); and *Commonwealth of Pennsylvania v. Kevin Foley*, 47 A.3d 882 (Pa. Super. 2012).

25. The TrueAllele calculation is entirely objective: when it determines the genotypes for the contributors to the mixture evidence, the computer has no knowledge of the comparison genotypes. Genotype comparison and match statistic determination are only done *after* genotypes have been computed. Moreover, the computer uses all the data, without user intervention. In this way, TrueAllele computing avoids human examination bias, and provides a fair match statistic.

26. I agree with the conclusions that were reached in the *Foley* case, which found that (i) scientists can validate the reliability of a computerized process even if the source code is not available to the public; (ii) it would not be possible to market TrueAllele if it were available for free; (iii) TrueAllele has been tested and validated.

TrueAllele is Considered to be Reliable

27. There is no genuine controversy as to the validity and reliability of the TrueAllele method. To the contrary, computer analysis of uncertain data using probability modeling is the scientific norm. Forensic science researchers see this as the best approach.

28. Cybergenetics thoroughly tests its software before it is released.

29. Over thirty-five validation studies have been conducted by Cybergenetics and other

groups to establish the reliability of the TrueAllele method and software. Eight of these studies have been published in peer-reviewed scientific journals, for both laboratory-generated and casework DNA samples. Source code was not needed or used in any of these studies.

30. In the "peer-review" process, scientists describe their research methods, results and conclusions in a scientific paper, which they submit to a journal for publication. An editor at the journal has (at least) two independent and anonymous scientists in the field read the paper, assess its merits, and advise on the suitability of the manuscript for publication. The paper is then accepted, rejected, or sent back to the authors for revision and another round of review.

31. A "laboratory-generated" validation study uses data that has been synthesized in a DNA laboratory, and is of known genotype composition. Five published TrueAllele papers of this type are: Perlin MW, Sinelnikov A. An information gap in DNA evidence interpretation. *PLoS ONE*. 2009;4(12):e8327; Ballantyne J, Hanson EK, Perlin MW. DNA mixture genotyping by probabilistic computer interpretation of binomially-sampled laser captured cell populations: combining quantitative data for greater identification information. *Science & Justice*. 2013;52(2):103-14; Perlin MW, Hornyak J, Sugimoto G, Miller K. TrueAllele® genotype identification on DNA mixtures containing up to five unknown contributors. *Journal of Forensic Sciences*. 2015;60(4):857-868; Greenspoon SA, Schiermeier-Wood L, and Jenkins BC. Establishing the limits of TrueAllele® Casework: a validation study. *Journal of Forensic Sciences*. 2015;60(5):1263-1276; Bauer DW, Butt N, Hornyak JM, Perlin MW. Validating TrueAllele® interpretation of DNA mixtures containing up to ten unknown contributors. *Journal of Forensic Sciences*. 65(2):380-398, 2020.

32. A "casework" validation study uses DNA data exhibiting real-world issues developed by a crime laboratory in the course of their usual casework activity. Three published TrueAllele papers of this type are: Perlin MW, Legler MM, Spencer CE, Smith JL, Allan WP, Belrose JL, Duceman BW. Validating TrueAllele® DNA mixture interpretation. *Journal of Forensic Sciences*. 2011;56(6):1430-1447; Perlin MW, Belrose JL, Duceman BW. New York State TrueAllele® Casework validation study. *Journal of Forensic Sciences*. 2013;58(6):1458-66; Perlin MW, Dormer K, Hornyak J, Schiermeier-Wood L, and Greenspoon S, TrueAllele® Casework on Virginia DNA mixture evidence: computer and manual interpretation in 72 reported criminal cases. *PLoS ONE*.

2014:9(3):e92837.

33. Conducting such validations is consistent with the FBI's 2010 Scientific Working Group on DNA Analysis Methods (SWGDAM) interpretation guidelines. TrueAllele complies with the 2015 SWGDAM validation guidelines for probabilistic genotyping systems. Regulatory bodies in New York and Virginia have had independent scientists review validation studies before they granted approval for their state crime laboratories to use TrueAllele for casework.

34. TrueAllele has been admitted into evidence after opposition challenge in twenty-nine courts, located in California, Florida, Georgia, Indiana, Louisiana, Massachusetts, Nebraska, New York, Ohio, Pennsylvania, South Carolina, Tennessee, Virginia, Washington, United States, Northern Ireland and Australia.

35. Twenty-seven admissibility decisions in the United States are: People of California v. Dupree Langston, Kern County (Kelly-Frye), BF139247B, January 10, 2013; State of Florida v. Lajayvian Daniels, Palm Beach County (Frye), 2015CF009320AMB, October 31, 2018; State of Georgia v. Adedoja Bah, Douglas Judicial Circuit (Harper), 17CR00938, October 16, 2019; State of Georgia v. Alexander Battle, Ben Hill County (Harper), 16-CR-082, May 22, 2019; State of Georgia v. Monte Baugh and Thaddeus Howell, Coweta County (Harper), 2017 R 618, March 11, 2019; State of Georgia v. Nathaniel Day, Tifton Judicial Circuit (Harper), 2018CR141, October 23, 2019; State of Georgia v. Thaddus Nundra, South Georgia Circuit (Harper), 18-CR-134, January 21, 2019; State of Georgia v. Guy Sewell, Floyd County (Harper), 17-CR-1675 JFL004, August 7, 2019; State of Indiana v. Randal Coalter, Perry County (Daubert), 62C01-1703-MR-192, August 2, 2017; State of Indiana v. Dugniqio Forest, Vanderburgh County (Daubert), 82D03-1501-F2-566, June 3, 2016; State of Indiana v. Vaylen Glazebrook, Monroe County (Daubert), 53C02-1411-F1-1066, February 16, 2018; State of Indiana v. Malcolm Wade, Monroe County (Daubert), 53C02-1411-F3-1042, August 3, 2016; State of Louisiana v. Chattley Chesterfield and Samuel Nicolas, East Baton Rouge Parish (Daubert), 01-13-0316 (II), November 6, 2014; State of Louisiana v. Harold Houston, Jefferson Parish (Daubert), 16-3682, May 19, 2017; State of Louisiana v. Kyle Russ, East Baton Rouge Parish (Daubert), 01-14-0566, April 30, 2019; Commonwealth of Massachusetts v. Heidi Bartlett, Plymouth County (Daubert), PLCR2012-00157, May 25, 2016; State of Nebraska v. Charles Simmer, Douglas

County (Daubert), CR16-1634, February 2, 2018; People of New York v. John Wakefield, Schenectady County (Frye), A-812-29, February 11, 2015; People of New York v. Casey Wilson, Chemung County (Frye), 2013-331, May 1, 2019; State of Ohio v. David Mathis, Cuyahoga County (Daubert), CR-16-611539-A, April 13, 2018; State of Ohio v. Maurice Shaw, Cuyahoga County (Daubert), CR-13-575691, October 10, 2014; Commonwealth of Pennsylvania v. Kevin Foley, Indiana County (Frye); State of South Carolina v. Jaquard Aiken, Beaufort County (Jones), 20121212-683, October 27, 2015; State of Tennessee v. Demontez Watkins, Davidson County (Daubert), 2017-C-1811, December 17, 2018; Commonwealth of Virginia v. Matthew Brady, Colonial Heights County (Spencer-Frye), CR11000494, July 26, 2013; State of Washington v. Emanuel Fair, King County (Frye), 10-1-09274-5 SEA, January 12, 2017; United States v. Lenard Gibbs, Northern District of Georgia (Daubert), 1:17-CR-207, May 30, 2019.

36. The Pennsylvania Superior Court affirmed the Foley decision on February 15, 2012, 2012 PA Super 31, No. 2039 WDA 2009. The New York State Supreme Court affirmed the Wilson decision on August 15, 2019, 175 A.D.3d 158 (3d Dep't 2019). The Nebraska Supreme Court affirmed the Simmer decision on November 1, 2019, 302 Neb. 369.

37. Cybergenetics has a strong financial incentive to ensure the reliability of its widely used TrueAllele system.

38. Cybergenetics continually tests its software and conducts scientific validation studies to ensure TrueAllele's reliability. Source code is not used in validation studies.

39. Cybergenetics improved the speed, accuracy and generality of the user interface LR match statistic calculation in February of 2014. The previous LR estimate could understate the match statistic by around a factor of ten. Genotype computation was not affected. This change is described in Cybergenetics application note "TrueAllele® VUIer™ Likelihood Ratio Calculation."

Background on Software Source Code

40. People write a computer program in a programming language using "source code".

41. This source code is later translated into computer-readable "executable" software.

42. The source code details step-by-step human-readable instructions that describe to the computer and programmers how the program operates.

43. TrueAllele is written in MATLAB (for MATrix LABoratory), a high-level mathematical language for programming and visualizing numerical algorithms made by the MathWorks (Natick, MA).

44. Here is an example of MATLAB source code, simplified from a few lines of the MathWorks built-in "mhsample" function that performs Metropolis-Hastings statistical sampling:

```
U = log(rand(nchain,nsamples+burnin));
for i = 1-burnin:nsamples
   y = proprnd(x0);
   q1 = logproppdf(x0,y);
   q2 = logproppdf(y,x0);
   rho = (q1+logpdf(y))-(q2+logpdf(x0));
   Ui = U(:,i+burnin);
   acc = Ui<= min(rho,0);
   x0(acc,:) = y(acc,:);
   accept = accept+(acc);
end
```

45. Thus, source code is written in language that humans are capable of understanding, but only if they are fluent in reading, writing and interpreting the particular language that the program is written in.

46. TrueAllele has about 170,000 lines of computer source code, written by multiple programmers over two decades. The computer code is dense mathematical text. It can take hours for a person to read through even a few dozen lines of MATLAB to decipher what it does. Reading at ten lines per hour would entail eight and a half person-years to review all the source code.

47. In my opinion, it is wholly unrealistic to expect that reading through TrueAllele source code would yield meaningful information.

Why TrueAllele is a Trade Secret

48. People can easily copy a computer program if they have its source code.

49. Source code contains the software design, engineering know-how, and algorithmic

implementation of the entire computer program.

50. Cybergenetics has invested millions of dollars over two decades to develop its TrueAllele system, the company's flagship product. Although the technology is patented, the source code itself is not disclosed by any patent and cannot be derived from any publicly disclosed source. Patent protection is not automatic, and litigation can cost tens of millions of dollars.

51. Cybergenetics considers the TrueAllele source code to be a trade secret. Cybergenetics does not disclose the source code to anyone outside the company. In fact, the source code has never been disclosed. The source code is not distributed to most employees of Cybergenetics, and copies are not provided to individuals, businesses or government agencies that use or license the software.

52. The fact that the source code is kept secret provides Cybergenetics with a significant advantage over others who do not have access to the source code and do not have the programming know-how or are not willing to make the investment necessary to develop comparable software.

53. Cybergenetics operates in a highly competitive commercial environment.

54. In recent years, at least ten other groups have developed similar software.

55. There is keen interest from competitors to find out how to replicate TrueAllele. The TrueAllele software represents a technological breakthrough that has not been successfully replicated by any other company as of this date.

56. Disclosure of the TrueAllele source code trade secret would cause irreparable harm to the company, enabling competitors to easily copy the company's proprietary products and services.

57. Ownership of the TrueAllele program and source code provides Cybergenetics with an advantage over its competitors who do not know the proprietary code and could not legally duplicate it.

58. Cybergenetics takes reasonable measures to protect the secrecy of the source code. For example, all information relating to the source code is housed on secure computers.

59. TrueAllele's source code derives value from remaining secret, and it has never been disclosed to the public.

60. In contrast to so-called "open source" programs, for-profit companies generally do not

make their source codes available to the public. The relatively few companies that have an open source business model tend to operate in a very large market, utilize free programmer coding, conduct little innovation, and earn their main revenue by providing software services.

61. Commercial software programs are extensively validated while in development and before release and commercialization. By their nature, open source programs typically are not validated prior to release, because the process of perfecting software is costly. Open source forensic DNA analysis software programs tend to be relatively short programs consisting of several hundreds of lines of code that realistically can be reviewed by a human being.

62. Open source software suffers from a lack of version control and quality assurance, since any unrelated party can make code changes and release untested products. This chaotic development approach is in marked contrast to the more controlled reliability and versioning requirements of forensic software that is used in criminal proceedings.

Irremediable Risks of Source Code Disclosure

63. Third party review of source code can divulge proprietary trade secrets wholly unrelated to reliability, but valuable to competitors. Once a review results in a release of hard-earned engineering know-how, that disclosure cannot be reversed. The source code reviewer's knowledge can be written into other software systems, shared with interested parties, or sold for profit. There are no adequate remedies for redress once this proprietary information has been released.

64. The credibility and trustworthiness of retained witnesses who testify about the alleged need for forensic source code has been undermined in cross-examination (*New York v. Jaquan Collins*, *Pennsylvania v. Michael Robinson*). Permitting such individuals to see proprietary information that is immaterial to a case is not reasonable, nor is it in the interest of justice.

65. Protective orders for source code are sometimes used in expensive civil litigation for patent infringement, which is not germane to criminal proceedings. Protective orders may fail to protect valuable trade secrets, leading to unwanted disclosure of proprietary designs, methods, and know-how (*Superspeed LLC v. Google*, United States District Court for the Southern District of Texas; *Bradford Technologies, Inc. v. NCV Software.com*, United States District Court for the Northern District of California; *Apple v. Samsung*, United States District Court for the Northern

District of California; *Eli Lilly & Co. v. Gottstein*, United States Court of Appeals for the Second Circuit; *Smith & Fuller, PA v. Cooper Tire & Rubber Co.*, United States Court of Appeals for the Fifth Circuit).

66. There is no real effective remedy once a protective order is violated. Courts typically merely reimburse the fees that were incurred by the party whose secrets were revealed. In a case involving source code that is a trade secret, however, once the source code has been revealed in breach of a protective order, it generally loses its status as a trade secret. The genie can't be put back in the bottle, and reimbursement of legal fees does nothing to compensate for the loss of commercial value.

67. Cybergenetics uniquely provides accurate, objective, and neutral DNA identification information for criminal justice. TrueAllele DNA match results are used by both prosecution and defense for an unbiased statistical assessment of biological evidence. Crime laboratories rely on their validated TrueAllele systems for effective interpretation of complex DNA data. Jeopardizing the existence of Cybergenetics through a disclosure of its source code is unreasonable, and does not serve the interests of justice.

Why TrueAllele Source Code is Not Needed

68. Cybergenetics offers the TrueAllele software for license by crime labs and to other interested parties.

69. The company currently charges a base license fee of $40,000.

70. Individuals and companies can also submit samples to Cybergenetics for testing and analysis for a fee.

71. Cybergenetics provides opposing experts the opportunity to review the TrueAllele process, examine results, and ask questions. This review can be done in Cybergenetics's Pittsburgh office, or through an Internet Skype-like meeting. Cybergenetics regularly explains the system, and the results obtained in a case, to both prosecution and defense. This introduction to the TrueAllele method, the case data, and the application of the method to the data, is a logical first step in understanding how the system works. Source code is not necessary.

72. The TrueAllele method is inherently objective, since the computer determines

evidence genotypes without any knowledge of the comparison reference genotypes or user manipulation of DNA data. Hence there is no possibility of examination bias when determining genotypes from the DNA data. Match statistics, whether inclusionary or exclusionary, are calculated only afterwards by comparing evidence genotypes with reference genotypes. Source code is not needed to understand that the TrueAllele process is objective.

73. TrueAllele's reliability was established on the evidence in this case. The report and its supporting case packet described the system's sensitivity, specificity and reproducibility on the DNA evidence. The case packet gives the data and parameter inputs used in running the program in the case. The packet also includes a case-specific mini-validation study of reported TrueAllele match statistics, measuring match specificity by comparison with non-contributor genotypes. Source code is not needed to understand or interpret these materials.

74. Additional discovery material for this case was provided on an optical disc. The DVD contains documents related to TrueAllele's reliability, such as background reading, over thirty validation studies and publications, regulatory approvals, general acceptance, and admissibility rulings. There are tutorial videos that describe TrueAllele methods and explain how the system works, as well as continuing legal education talks. The VUIer™ software for reviewing TrueAllele results is provided (with both Windows and Macintosh installers), along with instructions and user manuals. Case-specific files (data, reports, PowerPoint, case packet, VUIer input) are disclosed, enabling a thorough expert review. Source code is not needed to access these materials, read the files, use the executable VUIer software, or examine the computer results.

75. Cybergenetics offers commercial services for validating DNA mixture interpretation methods. Any party can provide DNA validation data and obtain these services to assess TrueAllele reliability. Since TrueAllele is an objective process, and produces unbiased DNA identification results that do not "know" comparison genotypes during analysis, it is easy for Cybergenetics to perform these studies. Source code is not needed for obtaining these services.

76. TrueAllele processing is available on-line through Cloud computing. Therefore, the system's capability can be operated as an Internet service, without purchasing a product. Any party can operate TrueAllele on the Cloud, and process their own DNA case or validation data. Moreover,

Cybergenetics makes this TrueAllele Cloud capability available to opposing parties at no charge so that they can conduct their own testing. Source code is not needed for assessing TrueAllele reliability, which is done by testing the executable program on actual data.

77. Although the source code for TrueAllele is a secret, the methodology it employs and implements has been disclosed. Cybergenetics has published the core mathematics of TrueAllele's underlying mathematical model for over 20 years. These publications include scientific papers (1995, 2001, 2009, and 2011) and patent specifications (2000 and 2001). Cybergenetics provides a compilation of these mathematical methods in a single summary document. This information discloses TrueAllele's genotype modeling mechanism, and enables others to understand or replicate the basic method. Indeed, at least ten other groups have developed their own software that uses TrueAllele's linear mixture analysis approach. The source code is not necessary or helpful to understand or test the methodology or reliability of the analysis.

78. To my knowledge, source code is not generally made available for most other commercial software that is regularly used and relied upon in the area of forensic DNA identification. Such software includes Life Technology's "Genemapper ID" for generating and analyzing DNA data signals, the Federal Bureau of Investigation's "PopStats" for producing DNA match statistics or "CODIS" for maintaining a DNA database, and Microsoft "Excel" for conducting additional DNA data analysis. Source code is not needed to assess the reliability of these essential software programs, since they have all been tested and validated.

79. When TrueAllele source code discovery has been requested by an opposing party, no court has ever ultimately required its disclosure. The requesting parties have been unable to show why source code would be material, reasonable, and in the interest of justice. Courts have denied such discovery requests in California, Maryland, New York, Ohio, Pennsylvania, Virginia, and Washington, often providing written rulings (*California v. Martell Chubbs*, *New York v. John Wakefield*, *Ohio v. Maurice Shaw*, *Pennsylvania v. Chelsea Arganda and Chester White*, *Pennsylvania v. Kevin Foley*, *Pennsylvania v. Michael Robinson*, *Washington v. Emanuel Fair*). Source code was not needed in any of these cases.

I declare the above is true and correct under penalty of perjury under the law of the Commonwealth of Pennsylvania, executed this __ day of September 2020 in Pittsburgh, Pennsylvania.

By: _____

EXHIBIT 5

J-A10008-11

2012 PA Super 31

COMMONWEALTH OF PENNSYLVANIA, Appellee v. KEVIN JAMES FOLEY, Appellant	IN THE SUPERIOR COURT OF PENNSYLVANIA No. 2039 WDA 2009

Appeal from the Judgment of Sentence of June 1, 2009
In the Court of Common Pleas of Indiana County
Criminal Division at No(s): CP-32-CR-0001170-2007

BEFORE: PANELLA, SHOGAN, and COLVILLE*, JJ.

OPINION BY PANELLA, J. FILED: FEBRUARY 15, 2012

Appellant, Kevin James Foley, appeals from the judgment of sentence entered on June 1, 2009, by the Honorable William J. Martin, President Judge of the Court of Common Pleas of Indiana County, Criminal Division. After careful review, we affirm.

In the early morning hours of April 13, 2006, Dr. John Yelenic, a dentist living alone in Blairsville, Pennsylvania, was brutally assaulted and murdered in his home. After an eight-day jury trial, Foley, a Pennsylvania State Police Trooper who was living with Dr. Yelenic's estranged wife,[1] was

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* Retired Senior Judge specially assigned to the Superior Court.

[1] The Commonwealth refers to Dr. Yelenic's wife as his "soon-to-be ex-wife." Appellee's Brief, at 37. However, Dr. Yelenic and his wife were married at the time of the murder, and representatives of Dr. Yelenic's estate were unable to obtain a posthumous divorce. ***See Yelenic v. Clark***, 922 A.2d 935, 936 (Pa. Super. 2007).

found guilty of first-degree murder and sentenced to life imprisonment. This timely appeal followed.

Appellant presents the following issues for our review:

I. WHETHER THE TRIAL COURT ERRED IN PRECLUDING THE TESTIMONY OF BETTY MORRIS AT TRIAL, WHERE THE EVIDENCE WAS RELEVANT AND ADMISSIBLE TO DEMONSTRATE THE MOTIVE OF ANOTHER PERSON TO COMMIT THE CRIME?

II. WHETHER THE TRIAL COURT ERRED IN ADMITTING THE TESTIMONY OF DR. MARK PERLIN, IN VIOLATION OF THE FRYE TEST FOR THE ADMISSIBILITY OF NOVEL SCIENTIFIC TESTIMONY?

III. WHETHER THE VERDICT WAS AGAINST THE WEIGHT OF THE EVIDENCE?

IV. WHETHER THE TRIAL COURT ABUSED ITS DISCRETION IN ADMITTING THE SHOE PRINT EVIDENCE AT TRIAL?

V. WHETHER THE TRIAL COURT ERRED IN INSTRUCTING THE JURY ON THE PERMISSIVE INFERENCE OF MALICE FROM THE USE OF A DEADLY WEAPON?

Appellant's Brief, at 4. We proceed to the merits.

Foley's first claim is that the trial court erred in excluding the testimony of Bette Morris.[2] The trial court may exercise its discretion in deciding whether to admit evidence, and our review of the trial court's evidentiary decisions is limited to determining whether the trial court abused

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[2] In his brief, Appellant refers to this witness variously as "Betty Morris," "Bette Morris," and "Bette Davis." Appellant's Brief, at 4, 23. This opinion will refer to her as Bette Morris, which is consistent with the notes of testimony and Appellee's brief. ***See*** N.T., March 17, 2009, at 134, 141.

its discretion. ***See Commonwealth v. Moser***, 999 A.2d 602, 605 (Pa. Super. 2010). The trial court abused its discretion only if its ruling "reflects manifest unreasonableness, or partiality, prejudice, bias, or ill-will, or such lack of support to be clearly erroneous." ***Id***.

During the criminal investigation of this case, Bette Morris said to a law enforcement officer that on two occasions she had observed Dr. Yelenic engaged in intimate acts with his next door neighbor, Melissa Uss. According to Foley's counsel, if placed on the stand, Bette Morris would deny that she had ever made such observations, and then counsel would treat her as a hostile witness and impeach her with the statement she gave police. ***See*** N.T., March 17, 2009, at 135. When the Commonwealth objected that this evidence was irrelevant, Foley's counsel explained that it was intended to show that Melissa Uss's husband had a motive to kill Dr. Yelenic: "[A] jury could infer that somebody who was having a romantic affair with Dr. Yelenic, the husband might be inclined to do something and that is a fair inference from that." ***Id***., at 137. However, when the trial court asked whether the defense had any evidence that Melissa Uss's husband knew of the supposed intimate acts, defense counsel conceded that he had no such evidence. ***See***

id. According to the defense, Bette Morris's observations were made when Mr. Uss was in the military and not at home.[3] ***See id.***, at 135.

The trial court excluded the testimony of Bette Morris on the grounds that it was "a mere suggestion of motive and therefore irrelevant and inadmissible." Opinion and Order of Court, November 4, 2009, at 10. Generally, "proof of facts showing the commission of the crime by someone else is admissible." ***Commonwealth v. Boyle***, 368 A.2d 661, 669 (Pa. 1977). However, the Pennsylvania Supreme Court has held that facts suggesting that someone had a motive should not be considered by the jury if the person had no knowledge of the suggestive facts. ***See Commonwealth v. Giovanetti***, 19 A.2d 119, 125 (Pa. 1941).

In ***Giovanetti***, the murder victim had an employer-provided life insurance policy with his wife, the defendant, listed as the beneficiary. ***See id***. The trial court refused the defendant's request to instruct the jury that it could consider the insurance policy as evidence of her motive only if it found that she knew about the policy before the murder. ***See id***. The Supreme Court reversed, holding that the wife's knowledge of the policy was necessary for it to be considered as evidence of her motive to kill. ***See id***.

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[3] Although Foley called Melissa Uss as a witness, he did not ask her any questions regarding the alleged romantic relationship with Dr. Yelenic. ***See*** N.T., March 16, 2009, at 106-15. Foley did not call her husband as a witness.

The trial court's decision to preclude the testimony of Bette Morris had a sufficient basis in the governing law and was not an abuse of discretion. Although intimate contact between the victim and Melissa Uss may *suggest* that her husband had a motive, "merely suggesting that someone else may have had a motive is not evidence." ***Commonwealth v. Rivers***, 644 A.2d 710, 715 (Pa. 1994). The trial court acted within its discretion in rejecting the testimony as irrelevant because the husband had no knowledge of the intimate contact. ***See Giovanetti***, 19 A.2d at 125. Because there was no other evidence corroborating the suggestion that Mr. Uss was a killer motivated by jealousy, the trial court's decision to preclude the testimony of Bette Morris was a permissible exercise of discretion.

Foley's reliance on ***Commonwealth v. Ward***, 605 A.2d 796 (Pa. 1992), is misguided. In that case, the defendant was a police informant who was convicted of arson. The trial court precluded evidence that the people whom he had informed against had threatened him and had committed the arson in retaliation against him. ***See id.***, at 797. In addition, the trial court precluded testimony from "an American Red Cross worker as to appellant's request for assistance following the fire, the organization's investigation, and its subsequent provision of emergency fund vouchers for clothing," which the defendant sought to introduce in order to "undermine the Commonwealth's evidence of motive by arguing the unlikelihood that appellant would destroy

all of his own worldly possessions merely because of a disagreement with his brother." ***Id***.

Ward is distinguishable from the instant case. In ***Ward***, the defendant's offer of proof indicated that the other potential perpetrators *knew* that the defendant had given information about them to the police. ***See id***. Further, the precluded evidence from the Red Cross worker concerned the defendant's own motive to commit the crime rather than someone else's motive. Unlike the testimony at issue in the instant case, the evidence at issue in ***Ward*** was relevant, and its exclusion violated the defendant's fundamental right to introduce relevant, admissible evidence. ***See id***. (citing ***Chambers v. Mississippi***, 410 U.S. 284 (1973)).

Foley's next claim is that the trial court erred in admitting the DNA-related testimony of Dr. Mark Perlin. A sample containing DNA from the victim and another person was found underneath the fingernail of the victim. This mixed sample was tested in a laboratory at the FBI, and three experts – Dr. Perlin, Dr. Robin Cotton, and Jerrilyn Conway, an FBI forensic scientist – used the FBI's data in developing their testimony. Each of the experts determined that Foley's DNA profile was consistent with DNA found in the sample. The experts differed in their estimates of the probability that someone other than Foley would possess DNA matching the DNA found in the sample – Conway testified that the probability that another Caucasian could be the contributor was 1 in 13,000; Dr. Cotton testified that the

probability was 1 in 23 million; and Dr. Perlin testified that it was 1 in 189 billion.

As with other evidentiary decisions, the trial court may exercise its discretion in deciding whether to admit expert testimony. ***See Commonwealth v. Ventura***, 975 A.2d 1128, 1140 (Pa. Super. 2009). The trial court's decision will be reversed only if the appellate court finds an abuse of discretion or an error of law. ***See id***.

Foley claims that Dr. Perlin's testimony is inadmissible because it fails the ***Frye***[4] test for the admissibility of scientific evidence. ***See*** Appellant's Brief, at 31. Pennsylvania continues to adhere to the ***Frye*** test, which provides that "novel scientific evidence is admissible if the methodology that underlies the evidence has general acceptance in the relevant scientific community." ***Betz v. Pneumo Abex LLC***, 998 A.2d 962, 972 (Pa. Super. 2010) (en banc) (citing ***Grady v. Frito-Lay, Inc.***, 839 A.2d 1038 (Pa. 2003)). The ***Frye*** test is a two-step process. ***See id***. First, the party opposing the evidence must show that the scientific evidence is "novel" by demonstrating "that there is a legitimate dispute regarding the reliability of the expert's conclusions." ***Id***. If the moving party has identified novel scientific evidence, then the proponent of the scientific evidence must show that "the expert's methodology has general acceptance in the relevant

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[4] ***Frye v. United States***, 293 F. 1013 (D.C. Cir. 1923).

scientific community" despite the legitimate dispute. ***Id***. (internal quotation marks omitted).

The trial court did not expressly determine whether Dr. Perlin's testimony was "novel scientific evidence." Opinion and Order of Court, March 3, 2009, at 2-3. Instead, the court found that Dr. Perlin's methodology was a refined application of the "product rule," a method for calculating probabilities that is used in forensic DNA analysis. ***See id***., at 2. The Pennsylvania Supreme Court has held that scientific evidence based on the product rule is admissible in the Commonwealth. ***See Commonwealth v. Blasioli***, 713 A.2d 1117, 1118 (Pa. 1998). Because Dr. Perlin's calculations were made using newer technology, the trial court rhetorically asked "at what point does the use of the product rule become novel science." Opinion and Order of Court, March 3, 2009, at 2. The trial court went on to find that Dr. Perlin's methodology was generally accepted. ***See id***., at 3, 5.

We find that Dr. Perlin's testimony was not "novel" as that term is defined in the governing law, and thus the trial court did not abuse its discretion in admitting the testimony. The "novelty" of scientific testimony turns on whether "there is a legitimate dispute regarding the reliability of the expert's conclusions," which is not necessarily related to the newness of the technology used in developing the conclusions. ***Betz***, 998 A.2d at 972. In ***Betz***, the court noted that novelty "is not restricted to new science," and "even 'bedrock' scientific principles may be subject to a ***Frye*** analysis" if

those principles become disputed. ***Id.***, at 973-74. Conversely, where there is no dispute, ***Frye*** should be "construed narrowly so as not to impede admissibility of evidence that will aid the trier of fact in the search for truth." ***Id.***, at 972.

Here, we find no legitimate dispute regarding the reliability of Dr. Perlin's testimony. Dr. Perlin used proprietary software called TrueAllele to interpret the data he received from the FBI. ***See*** N.T., March 12, 2009, at 130. Foley claims that Dr. Perlin's testimony should have been excluded for three reasons: (1) "as of the date of the pre-trial hearing, no forensic laboratory in the United States used Perlin's TrueAllel [sic] method in analyzing a mixed sample of DNA for forensic purposes"; (2) "the TrueAllel [sic] system had never been used in a court of law in any jurisdiction in the United States on a mixed DNA sample to give a likelihood ratio"; and (3) no outside scientist can replicate or validate Dr. Perlin's methodology because his computer software is proprietary. Appellant's Brief, at 35.

Foley's first claim does not amount to a showing of "novelty" because it does not show a "legitimate dispute regarding the reliability of the expert's conclusions." ***Betz***, 998 A.2d at 972. Regardless, Foley understates the extent of usage of Dr. Perlin's system. As Dr. Perlin testified:

> The TrueAllele technology is used by New York State for all of their data banking and bringing their casework system on board. The Allegheny County Crime Lab has been using our system as a service and recently purchased the system for looking at mixtures in complex cases and DNA evidence. The World Trade Center engaged us to reanalyze all of the data and rematch it

> using our methods from the eighteen thousand (18,000) or so victim remains and the three thousand (3000) missing people and so on and there are other groups that we work with.

N.T., Mar. 12, 2009, at 132.

In addition, the United Kingdom's Forensic Science Service uses TrueAllele technology to analyze crime scene evidence and build the UK National DNA Database, which is the largest of its kind in the world. ***See*** Forensic Science Service Expands License for Cybergenetics Automated DNA Data Review Technology; Pioneering TrueAllele Software Helps Builds [sic] World's Largest DNA Database, Business Wire, July 26, 2004, available at http://tinyurl.com/8yxh8hd (last visited Nov. 21, 2011); ***see also*** Opinion and Order of Court, March 3, 2009, at 5.

Foley's second reason for excluding the testimony is not persuasive because "novelty" of a scientific methodology does not turn on its previous use in court. During cross-examination, Dr. Perlin testified that he did not know whether any users of TrueAllele had used it in a case that went to trial. ***See*** N.T., March 12, 2009, at 133-34. Even if Foley is correct that TrueAllele has never been used in court, this would not prove novelty. The Commonwealth's "continued adherence to the ***Frye*** test is based upon its interest in having judges be guided by scientists when assessing the reliability of a scientific method, and not the other way around." ***Betz***, 998 A.2d at 979 (internal quotation marks omitted). If this court assessed "novelty" of scientific evidence based on its previous use in court, we would

be failing to defer to scientists in assessing the reliability of scientific methods. Rather than looking to previous uses in court, we find "novelty" only if there is a dispute among scientists. ***See Betz***, 998 A.2d at 972.

Foley's third reason for exclusion is misleading because scientists can validate the reliability of a computerized process even if the "source code" underlying that process is not available to the public. TrueAllele is proprietary software; it would not be possible to market TrueAllele if it were available for free. ***See*** N.T., Hearing, February 18, 2009, at 54. Nevertheless, TrueAllele has been tested and validated in peer-reviewed studies. One study used laboratory-generated DNA samples and found that quantitative analysis performed by TrueAllele was much more sensitive than qualitative analysis such as that performed by the FBI. ***See*** Perlin & Sinelnikov, An Information Gap in DNA Evidence Interpretation, 4 PLoS ONE e8327, at 10 (2009), available at http://dx.doi.org/10.1371/journal.pone.0008327. A recent paper entitled "Validating TrueAllele® DNA Mixture Interpretation" used DNA samples from actual cases and reached similar results. ***See*** Perlin et al., Validating TrueAllele® DNA Mixture Interpretation, 56 Journal of Forensic Sciences 1430 (2011). The study "validated the TrueAllele genetic calculator for DNA mixture interpretation" and found that "[w]hen a victim reference was available, the computer was four and a half orders of magnitude more

efficacious than human review."[5] ***Id.***, at 1444. Both of these papers were published in peer-reviewed journals; thus, their contents were reviewed by other scholars in the field.

Because Foley has failed to establish the existence of a legitimate dispute over Dr. Perlin's methodology, he has failed to show that Dr. Perlin's testimony constituted "novel" scientific evidence. ***See Betz***, 998 A.2d at 972. Therefore, we find that the trial court's decision to admit the testimony was not an abuse of discretion. Absent a legitimate dispute, there is no reason to "impede admissibility of evidence that will aid the trier of fact in the search for truth." ***Id.***

Foley's next claim is that the trial court abused its discretion when it admitted evidence related to bloody shoeprints found at the murder scene. Foley claims that a new trial should be awarded because this evidence was irrelevant and highly prejudicial. ***See*** Pa. R. Evid. 402, 403. As noted above, this court will find an abuse of discretion only if the trial court's ruling "reflects manifest unreasonableness, or partiality, prejudice, bias, or ill-will, or such lack of support to be clearly erroneous." ***Commonwealth v. Moser***, 999 A.2d 602, 605 (Pa. Super. 2010).

Foley claims the shoeprint evidence was irrelevant because "[t]he shoe prints found at the scene could not be authoritatively determined to be any

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[5] In this case, a victim reference was available because the evidence was taken from the victim's fingernail. ***See*** N.T., March 12, 2009, at 89.

particular brand, style, or size of shoe." Appellant's Brief, at 61. At trial, the Commonwealth introduced expert testimony from an FBI forensic examiner that the shoeprints at the crime scene apparently were left by an Asics brand running shoe with the model name "Gel Creed" or "Gel Creed Plus." N.T., March 13, 2009, at 45. The FBI forensic examiner noted that he could not state his opinion with one hundred percent certainty because the FBI database does not contain reference information for every shoe manufactured in the world. ***See id***., at 47

The Commonwealth also introduced testimony from Terry Schalow, a product manager for Asics America Corporation. He testified that the shoeprint was left by an Asics Gel Creed, Gel Creed Plus, or a knockoff of this type of shoe. ***See id***., at 18-19. The size was between ten and twelve and a half. ***See id.***, at 18. Only about 25,000 Gel Creed shoes were sold in the United States. ***See id***., at 20. Importantly, Foley ordered a size ten Gel Creed from Asics in August 2003. ***See id***., at 25, 27.

Contrary to Foley's position, the uncertainty in this testimony goes to its weight rather than its admissibility. Foley emphasizes that neither expert could state with absolute certainty that the shoeprints were left by size 10 shoes manufactured by Asics and purchased by Foley. However, to be relevant and admissible, "evidence need not be conclusive." ***Commonwealth v. Crews***, 640 A.2d 395, 402 (Pa. 1994). Evidence is relevant if it logically tends to establish a material fact in the case or tends

to support a reasonable inference regarding a material fact. ***See id***. Here, the shoeprint evidence supported a reasonable inference that Foley was at the scene of the crime. This relevant, though inconclusive, evidence was admissible, and "its weight and persuasiveness were properly matters for the jury to determine." ***Id***., at 403.

Foley's charge that the shoeprint evidence was "highly prejudicial" is also not persuasive. ***See*** Appellant's Brief, at 64. The Pennsylvania Rules of Evidence provide that "[a]lthough relevant, evidence may be excluded if its probative value is outweighed by the danger of **unfair** prejudice, confusion of the issues, or misleading the jury, or by considerations of undue delay, waste of time, or needless presentation of cumulative evidence." Pa. R. Evid. 403 (emphasis added). Evidence is not unfairly prejudicial simply because it is harmful to the defendant's case. ***See Commonwealth v. Page***, 965 A.2d 1212, 1220 (Pa. Super. 2009). Rather, exclusion of evidence on this ground "is limited to evidence so prejudicial that it would inflame the jury to make a decision based upon something other than the legal propositions relevant to the case." ***Id***. While the shoeprint evidence tended to support an inference that Foley committed the crime, there is no reason to believe that it improperly inflamed the jury. Thus, the trial court did not abuse its discretion by admitting the shoeprint evidence.

Next, we turn to Foley's claim that the jury's verdict was against the weight of the evidence. Foley preserved this claim for appellate review by

raising it with the trial judge in a post-sentence motion. ***See*** Pa. R. Crim. P. 607; ***see also*** Opinion and Order of Court, November 4, 2009, at 1. Our standard of review is well-settled:

> The finder of fact is the exclusive judge of the weight of the evidence as the fact finder is free to believe all, part, or none of the evidence presented and determines the credibility of the witnesses.
>
> As an appellate court, we cannot substitute our judgment for that of the finder of fact. Therefore, we will reverse a jury's verdict and grant a new trial only where the verdict is so contrary to the evidence as to shock one's sense of justice. A verdict is said to be contrary to the evidence such that it shocks one's sense of justice when "the figure of Justice totters on her pedestal, or when "the jury's verdict, at the time of its rendition, causes the trial judge to lose his breath, temporarily, and causes him to almost fall from the bench, then it is truly shocking to the judicial conscience."
>
> Furthermore,
>
> where the trial court has ruled on the weight claim below, an appellate court's role is not to consider the underlying question of whether the verdict is against the weight of the evidence. Rather, appellate review is limited to whether the trial court palpably abused its discretion in ruling on the weight claim.

Commonwealth v. Cruz, 919 A.2d 279, 281-82 (Pa. Super. 2007) (citations omitted).

We find that the trial court did not abuse its discretion in finding that the verdict was not against the weight of the evidence. Over the course of the eight-day trial, copious evidence linking Foley to the crime was presented to the jury. This evidence was comprehensive and credible enough to support the verdict.

At the time of the murder, Foley was living with Dr. Yelenic's estranged wife. Foley had expressed his hatred of Dr. Yelenic to numerous individuals – Foley had said that he wished Dr. Yelenic would die, and on one occasion Foley asked a fellow police officer to help him kill Dr. Yelenic. On three occasions, Foley attempted to have Dr. Yelenic investigated and arrested for child abuse, and Foley was frustrated by his lack of success.

Foley had an opportunity to commit the crime. At the approximate time of the murder, he was driving from a hockey game in Delmont to his home in Indiana, which took him past Blairsville, where Dr. Yelenic resided.

Foley's DNA profile was consistent with DNA found under Dr. Yelenic's fingernail, and the most conservative estimate of the likelihood that someone else would possess a consistent profile was one in 13,000.[6] On the night before the murder, Foley had no abrasion on his forehead, but on the morning following the murder he had an injury on his forehead described by three eyewitnesses as "a fingernail scratch" and by others as a cut that appeared to be "fresh."

The shoeprint evidence, discussed above, supported a reasonable inference that Foley was present at the scene. Foley said that he did not remember what happened to the size 10 pair of Gel Creed shoes he ordered in 2003.

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[6] Foley does not challenge the reliability of the scientific methodology underlying this estimate.

Dr. Yelenic was slashed by a sharp instrument, and Foley was known by his colleague to be a "knife guy" who habitually flicked open and shut a knife that he carried with him. In fact, Foley once accidentally sliced open a supervisor's pair of pants in the groin area when he was walking past him. When informed of Dr. Yelenic's death shortly after the discovery of the murder, Foley was unemotional, expressed no curiosity about the nature or cause of death, and only asked which law enforcement agency was in charge of the investigation. After the murder, Foley stopped playing with his knife and started wearing Nike brand shoes instead of Asics.

Given this evidence, the verdict is hardly shocking to the judicial conscience. The court below acted within the bounds of its discretion as the finder of fact. Thus, we reject Foley's claim that the verdict was against the weight of the evidence.

Finally, we turn to Foley's argument that the trial court erred in instructing the jury on the permissive inference of malice from the use of a deadly weapon. The trial court instructed the jury that "[i]f you believe that the defendant intentionally used a deadly weapon on a vital part of John J. Yelenic's body, you may regard that as an item of circumstantial evidence from which you may, if you choose, infer that the defendant acted with malice." N.T., March 18, 2009, at 229.

Foley concedes that the Supreme Court of Pennsylvania has approved this charge in a homicide case. ***See Commonwealth v. Jones***, 912 A.2d

268, 279-80 (Pa. 2006). Nevertheless, Foley argues that "this is an unconstitutional charge that deprived him of due process and should now be overruled." Appellant's Brief, at 65. However, this court has a "duty and obligation to follow the decisional law of [the Supreme Court of Pennsylvania]." ***Commonwealth v. Shaffer***, 734 A.2d 840, 844 n.6 (Pa. 1999). "The primary role of the Superior Court is to apply existing law to the cases that come before us. It is not our function to attempt reversing viable Supreme Court rulings . . . ." ***L.B. Foster Co. v. Charles Caracciolo Steel & Metal Yard Inc.***, 777 A.2d 1090, 1096 (Pa. Super. 2001).

Because the challenged jury instruction has been approved by the Supreme Court, we find that the trial court accurately instructed the jury on the law of the Commonwealth. ***See Jones***, 912 A.2d at 279-80. Accordingly, we reject Foley's claim and affirm the judgment of sentence.

Judgment of sentence affirmed. Jurisdiction relinquished.

Judgment Entered:

Eleanor R. Valecko

Deputy Prothonotary

DATE: FEBRUARY 15, 2012

EXHIBIT 6

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PAPER

CRIMINALISTICS

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Establishing the Limits of TrueAllele® Casework: A Validation Study

ABSTRACT: The limits of the expert system, TrueAllele® Casework (TA), were explored using challenging mock casework profiles that included 17 single-source and 18 two-, 15 three- and 7 four-person DNA mixtures. The sensitivity (ability to detect a minor contributor) of the TA analysis process was examined by challenging the system with mixture DNA samples that exhibited allelic and locus dropout and other stochastic effects. The specificity (ability to exclude nondonors) was rigorously tested by interrogating TA derived genotypes with 100 nondonor profiles. The accuracy with which TA estimated mixture weights of contributors to the two-person mixtures was examined. Finally, first-degree relatives of donors were used to assess the ability of the system to exclude close relatives. TA demonstrated great accuracy, sensitivity, and specificity. TA correctly assigned mixture weights and excluded nearly all first-degree relatives. This study demonstrates the analysis power of the TrueAllele® Casework system.

KEYWORDS: forensic science, DNA typing, expert system, DNA mixtures, probabilistic modeling, likelihood ratio

While even compromised single-source profiles typically lend themselves readily to human interpretation, mixture analysis poses a greater challenge for the forensic examiner. Forensic mixture samples, those biological specimens compromised of DNA from more than one individual, constitute a large proportion of casework samples. In fact, the level of sophistication and complexity of the analysis methods applied to DNA mixture sample interpretation has increased steadily, as has the complex nature of the sample types profiled by the forensic examiner (1–4). In 2010, the Scientific Working Group on DNA Analysis Methods (SWGDAM) recommended along with other guidelines that stochastic thresholds be applied to mixture samples (5). A stochastic threshold is designed to alert the DNA analyst that all of the DNA typing information may not have been detected for a given sample, that is, that there is potential for allelic dropout. Alternate statistical approaches were suggested to accommodate the uncertainty of the data.

Frequently, with the application of stochastic thresholds to DNA mixture sample electropherogram data, the combined probability of inclusion/exclusion (CPI/CPE) is rendered impotent as a means of expressing the statistical value of a profile due to loss of data below the stochastic threshold. Thus, it comes as no surprise that a number of software programs described as expert systems have been developed to assist the forensic examiner in performing scientifically based and statistically sound interpretations of the mixed contributor DNA evidence. Such an expert system would utilize much of the allele data that fall below the stochastic threshold (6–8). The testing and evaluation of one of these systems, TrueAllele® Casework (Cybergenetics, Pittsburgh, PA), is the subject of the study reported herein. This study was designed by and undertaken at the Virginia Department of Forensic Science (VDFS) to test the performance and define the limits of the TrueAllele® Casework expert system.

TrueAllele® Casework is a continuous probabilistic modeling system that utilizes Markov chain Monte Carlo (MCMC) sampling of the joint distribution, a probability distribution that combines all of the random variables, to perform an exhaustive statistical modeling of the electropherogram data (8,9). Probabilistic modeling as a means to deconvolve or solve a complex problem is not a new invention and has been successfully utilized by many diverse disciplines since its advent post-WWII (10). A wide range of disciplines such as nuclear physics, psychology, computer learning, economics, biological systems, and more recently, DNA analysis, utilize probabilistic modeling to make sense of the patterns observed in complex data and predict likely outcomes for various tests (11–13). Moreover, computer modeling can allow for the trialing of thousands or even millions of different explanations for the observed data using large numbers of variables within a time frame that escapes a purely human endeavor (14–17).

The TrueAllele® Casework system utilizes MCMC analysis in order to try many thousands of different combinations of variables to explain the DNA profile data. The short tandem repeat (STR) data are displayed in the form of an electropherogram generated as a final product of DNA profiling. Following Bayes' theorem, the observed data are separated into derived contributor genotypes which are used to update prior probability into posterior probability (9,18). TrueAllele® Casework can then answer the question of whether there is statistical support for or against the person of interest being a contributor to a mixture or single-source DNA profile. Moreover, this modeling of the data to generate derived contributor genotypes occurs prior to and independent of any comparison to the person of interest's reference

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profile and thus provides an objective and unbiased analysis of forensic DNA casework.

All testing of the system was performed at VDFS using compromised single-source profiles and challenging two-, three-, and four-person mixed donor DNA profiles created on site. The PowerPlex® 16 (Promega Corp., Madison, WI) STR profiles of all samples utilized had been documented and all genotypes were known. Thus, a critical assessment of software performance could be performed as all genotyping answers to the questions posed to the system were previously established. Moreover, samples were chosen to stress the system allowing for an evaluation of the performance of the modeling program when confronted with samples exhibiting allelic and locus dropout, artifacts and many alleles below the stochastic and analytical (limit of detection) thresholds. These are the very artifacts routinely encountered when performing DNA analysis on forensic casework.

The TrueAllele® Casework software system, like any other instrument, has limits. This body of work was designed to identify those limits and from that data, formulate policy and procedure for accurate and reproducible forensic DNA mixture analysis. The sensitivity (ability to detect trace donors), the specificity (ability to exclude nondonors), and the ability to exclude first-degree relatives to donors of a mixture were deemed most crucial to define, but other aspects to sample analysis were also evaluated.

Materials and Methods

DNA Sample Preparation

DNA samples were purified from previously collected dried blood and buccal samples obtained from volunteers. DNA was extracted using the DNA IQ® System (Promega Corp.) or organic purification, as described (19). All samples are listed in File S1. DNA samples were quantitated using the Plexor® HY System (Promega Corp.) and amplified using the PowerPlex® 16 System as described (19). Amplified samples were separated on the 3130*xl* Genetic Analyzer (Applied Biosystems, Foster City, CA) as described (20). Analysis was completed by the GeneMapper® ID v3.2.1 software (ABI). The stutter cutoffs were defined as well as the limit of detection (LOD; blue 73, green 84, yellow 75, and red 52 RFUs) (20).

Electropherogram data (.fsa files) were utilized from previously analyzed single-source and mixture DNA samples as described in the sample preparation section above. Challenging single-source profiles were obtained from amplified DNA used for establishing stochastic thresholds and for environmental studies. Two samples with a high degree of heterozygosity originally used to establish the stochastic threshold were analyzed by TrueAllele® Casework (two 30 pg samples and three 10 pg samples from sample S9; three 30 pg samples and two 10 pg samples from sample S1). The profiles subjected to stochastic effects from the two different donors were compared to both donor S9 and S1 reference profiles to generate a match statistic. Eight degraded samples from three different donors (S1, S11, and S3) were analyzed using TrueAllele® Casework and then compared to the reference profile for the donor and ten nondonors to generate the match statistic.

Eighteen two-person mixture samples were obtained from previously analyzed mixture studies as well as mock casework. The mixtures samples were derived from different combinations of donors and by differing the ratios of DNA from the donors. A total of five different samples were used to create the eighteen two-person mixture profiles. Fifteen three-person mixture samples were obtained from previously analyzed mixture studies and mock casework samples. A total of seven different donors were used to create the three-person mixture profiles. As with the two-person mixtures, the profiles were derived from different combinations of donors and by differing the ratios of DNA from the donors. Seven four-person mixture samples were obtained from previously analyzed mixture studies. A total of eight different donors were used to create the four-person mixture profiles and they also consisted of different combinations of donors and differing ratios of the DNA from the donors. For all analyses except for the specificity tests, eleven reference profiles (S1–S11) were used for comparison and generation of the match statistics. All donors used to create the mixture samples were contained within the set of 11 reference profiles. The reference samples were previously typed using the PowerPlex® 16 System and uploaded to TrueAllele® Casework by manually entering them as text files.

The TrueAllele® Casework System

TrueAllele® Casework (TA) is a genotype modeling system that uses probability to define the most likely explanations for the data. TA uses MCMC analysis to examine many different variables in order to account for the observed data (with STR data, a sample's electropherogram peak height and molecular weight data) (8,9,17). Variables such as genotype and mixture weight (each contributor's proportion in the mixture), among others, are mathematically combined in probability equations modeled to explain the data.

Each TA cycle sequentially tests the variables to accept or reject values. When TA proposes a new value for a variable, it compares the joint probability (of data and model) using that new value relative to the old probability. For example, a cycle might compare the probability of a 50:50 mixture ratio for a two-person mixture relative to that of a 55:45 ratio. When the joint probability is higher, the new value is accepted.

The reported cycle numbers (25K, 50K, 100K, or 200K) refer to the number of times TA sequentially tests all of the variables (25K refers to 25,000 cycles, 50K refers to 50,000 cycles, etc.). For this study, the same cycle value was utilized for both "burn-in" and "read-out". The "burn-in" phase moves the system into the posterior probability region (e.g., mixture weight values that better explain the data). In the MCMC "read-out" phase, the system statistically samples from that region (e.g., determining the mixture weight probability distribution). TA analyses of a sample run for different cycle numbers can still be concordant and are evaluated using the same metrics. Some complex mixtures will be better resolved using a greater number of cycles, but running mixture samples longer typically impacts minor contributors much more than more predominant contributors (pers. obs.).

Production of the Match Statistic

After the derived contributors are produced by the TA software system, a comparison is performed between the derived contributors of a sample and reference samples of interest selected by the user. The comparison is in the form of a likelihood ratio (LR) and also referred to as the match statistic. This value can also expressed as a logarithm of the LR, log(LR) (20,21). The match statistic for a comparison with a particular reference profile is the log(LR) which is the highest, most discriminating value for that analysis of the evidence sample. A

positive match statistic is where the log(LR) is positive, which means the LR is above one. A comparison which provides no statistical support for a match is where the log(LR) is negative, which means the LR is below one. Concordant analyses should produce match statistics that are within two log units (ban) of each other. For the study, a positive log(LR) is referred to as an inclusion and a negative log(LR) is referred to as an exclusion.

TrueAllele® Casework analyses were performed at 25, 50, and 100K cycles for the stochastic samples and at 25K twice and at 100K once for the degraded samples, except for one sample, S11 (UV treated for 3 months), which was analyzed once at 25K and twice at 100K cycles. TA analyses for the two-, three-, and four-person mixtures ranged from 25K to 200K, although 25K was determined to provide inadequate sampling for the complex three- and four- person mixtures and was discontinued.

Operation of the System

The operation of the TrueAllele® Casework system (server v. 3.25.4441.1, VUIer v. 3.3.5148.1) was performed as described in the TrueAllele® VUIer™ manuals (22). Also utilized was the information and training provided by Cybergenetics for both Operator I and Operator II level training courses and in the literature. A theta correction value of 0.01 was employed for all analyses using VDFS allele frequencies.

Single-source profiles from degraded (7) and low template samples that exhibit stochastic effects (10) were analyzed using TrueAllele® Casework and compared to the donor reference as well as nondonor reference profiles. Two-, three-, and four-person mixtures were subjected to the TrueAllele® Casework analysis process and compared to a series of eleven reference profiles (named S1–S11 and including the true donors) for generation of the log(LR) match statistic. All single-source, mixture, and reference profile compositions are listed in File S1.

Evaluation of Data Output

The data produced by the TrueAllele® Casework system were evaluated for metrics listed in Table 1. The quality of the analysis which included the Markov chain sampling, the Gelman–Rubin convergence statistic value {≤1.2, >1.2 and ≤1.5, >1.5} and histogram of derived mixture weights, was the initial quality aspects of the TA analysis evaluated after the software completed the deconvolution process (23). The MCMC provides a visible record and history of the statistical sampling of mixture weights for an analysis. Figs 1 and 2 display two independent analyses of a complex three-person mixture, Mix3_6. Fig. 1 depicts an ideal analysis and Fig. 2 depicts a nonideal (poor) analysis, given the complexity of the mixture.

TABLE 1—*Metrics assessed for TrueAllele® Casework Analysis. The three metrics listed are the first aspects to be assessed for a deconvoluted mixture sample.*

Metrics	Ideal	Acceptable	Poor
Markov chain (MC)	Good sampling of the "space". MCs with minimal/ no sampling for no more than ~20% of sampling time	MCs with minimal/no sampling for more than ~20% of sampling time	MCs stuck with no sampling. Rope-like appearance of the chain
MW Histogram	SD > 0.03 for complex mixtures	SD ≥ 0.03 for complex mixtures	SD < 0.03 for complex mixtures
Gelman-Rubin Convergence	≤1.2	≤1.5	>1.5*

MW, Mixture weight; SD, standard deviation.

*In some concordant samples, an analysis with poor convergence values may still be used for reporting.

After the initial assessment of the run metrics described above, other characteristics of the analyses were evaluated. The reproducibility of the results (genotype concordance and similar match statistics) was assessed. Reproducible match statistics were defined as a minimum of two ideal or acceptable analyses with the log(LR)'s within 2 ban (log units). Also assessed was if the correct individuals were included (generated a positive match statistic) and if nondonors were excluded (generated a negative match statistic). The derived mixture weights for concordant analyses should be similar, but do not need to be exact. For mixtures with very minor contributors (less than 15%), the mixture weights for the more minor contributors may show increased variability, even for concordant, ideal analyses. The Kullback–Leibler (KL) statistic (the information content of a derived contributor genotype) was also evaluated; however, it was not used for any determinations of concordance (24).

An example of good genotype concordance versus poor genotype concordance is shown in Fig. 3 for independent analyses of the same complex three-person mixture, Mix3_6. Excellent genotype concordance is depicted in Panel (a) with the predominant derived contributor for both the ideal and poor (nonideal) analyses. The correct genotype of the predominant contributor to the mixture is circled. Poor genotype concordance for the most minor derived contributor genotype (the correct minor contributor genotype is circled) is observed between the ideal and poor analyses. Genotype concordance that is deemed "fair" will typically fall between the two extremes of poor and good, showing good concordance at many loci and poorer concordance at other loci. The probability value assigned for each genotype is also considered when assessing the quality of the genotype concordance as more concordant genotypes typically display more similar genotype probabilities.

Typically for a poor TA analysis, the predominant derived contributor is concordant with the predominant derived contributor for an ideal analysis; however, a very minor contributor, as was the case for Mix3_6, may not be captured by the MCMC sampling process (personal obs.). As shown in Fig. 3, a lack of concordance was observed between the most minor derived contributor for the ideal and the poor analyses. A 100% probability for a nonconcordant genotype was produced for the most minor derived contributor for the poor analysis, whereas a distribution of genotypes was produced for the ideal analysis. The true genotype of the most minor contributor was included in that distribution for the ideal analysis (6,9.3). Thus, the poor analysis failed to capture the most minor contributor to the mixture due to insufficient sampling.

Mixture Ratio Assessment

Mixture weights for two-person mixtures were initially estimated based upon quantitation data and the input ratios of the quantitated DNA placed into the PowerPlex® 16 System amplification reaction. After generation of the electrophoretic data, manual estimates were created using loci for which there was no allele sharing between contributors (loci with four alleles visible or loci with two minor alleles and one major allele). The peak

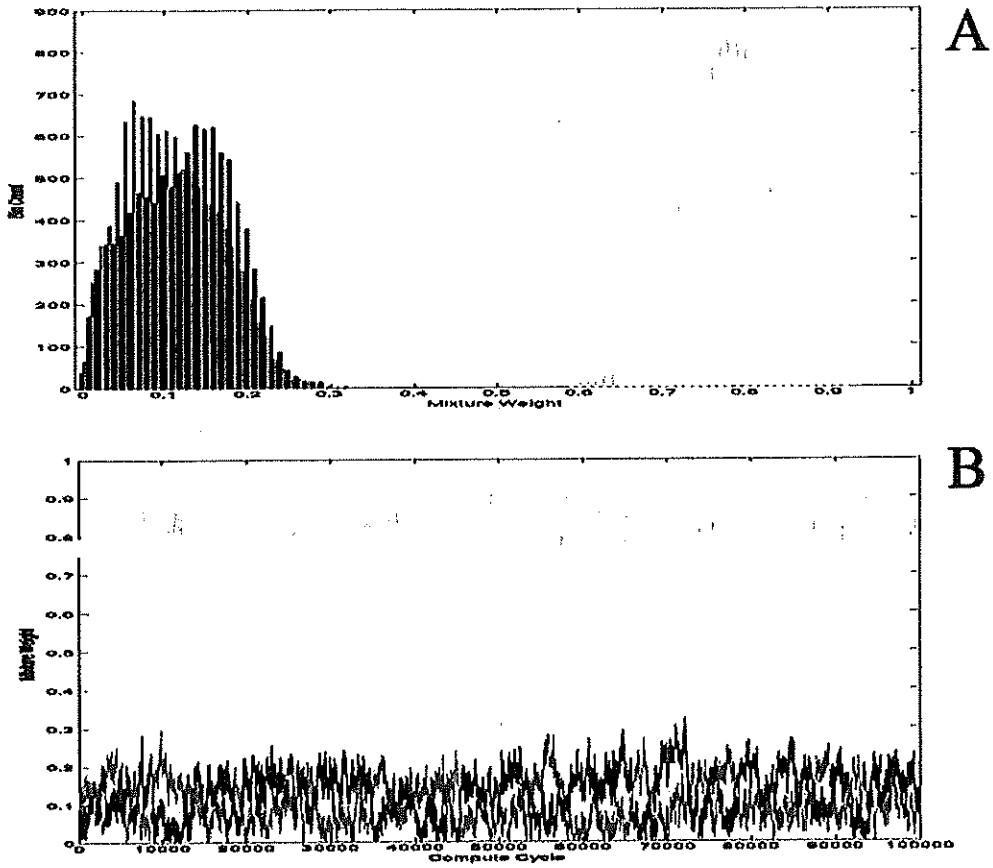

FIG. 1—*Markov chain and histogram of an ideal analysis of the complex three-person mixture, Mix3_6. (Panel A) Histogram of derived mixture weights for the three-person mixture. (Panel B) The corresponding ideal Markov chain history of the mixture weight sampling. The three colors indicate each derived contributor.*

height values for the minor alleles were summed and divided by the sum of the peak heights for all of the alleles.

Specificity of the TrueAllele® Casework System

Specificity, the ability to exclude noncontributors, of the TrueAllele® Casework analysis process was evaluated. The derived contributor genotypes of two-, three-, and four-person mixture samples were utilized for the test. Only ideal or acceptable TrueAllele® Casework analyses that were retained and used for genotype concordance were utilized for comparison with reference profiles.

All of the derived contributor genotypes from the two-, three-, and four-person mixture profiles were interrogated for the match statistic using 100 synthetically generated PowerPlex® 16 profiles kindly provided by Cybergenetics. None of the 100 profiles were donors to any of the mixtures tested. To form the reference profiles, a computer randomly sampled allele pairs at each locus from a representative human allele count database. The random profiles were saved as text files for subsequent upload to a TrueAllele® World and eventual match comparison.

The TrueAllele® Casework system allows the user to manage data in virtual worlds. A TA world will contain the STR data, interpretation requests and the MCMC joint distributions. The Cybergenetics representative population database (named CYB) is a multi-ethnic allele count database based on five thousand anonymous individuals (M. Legler, Cybergenetics, pers. comm.). The synthetically derived PowerPlex® 16 profiles were uploaded to TrueAllele® Casework as text files. Match statistics were performed for all three major population groups: Black, Caucasian, and Hispanic.

The ability of the TA system to distinguish relatives versus true donors to the two-, three-, and four-person mixture samples was assessed. Only first-degree relatives were tested, therefore, "sons" were manually created from seven of eleven reference profiles by selecting one of the reference profile alleles at each locus and randomly selecting a sister allele to create a "son". Of the eleven reference samples used for this validation study, ten of those were donors used for creation of the two-, three-, and four-person mixtures. Of the seven profiles that were used to synthesize "sons", six were donors to the two-, three-, and four-person mixtures. Match statistics for the mixture profiles were generated for all of the eleven reference profiles as well as the seven "sons".

Additionally, "brothers" were manually created from five profiles of donors to the two-, three-, and four-person mixtures. This was carried out by estimating the expected ratios given a sibling relationship of both alleles being shared, one allele shared and no alleles shared. The siblings were created in this manner to ensure that they shared many alleles and thus would challenge the TrueAllele® Casework system. Furthermore, the profiles of the references and the "brothers" were entered into Popstats (a module of the Federal Bureau of Investigation's CODIS software) to calculate a sibling index. All sibling indices surpassed the minimum of 33 used as an inclusion threshold at VDFS (25).

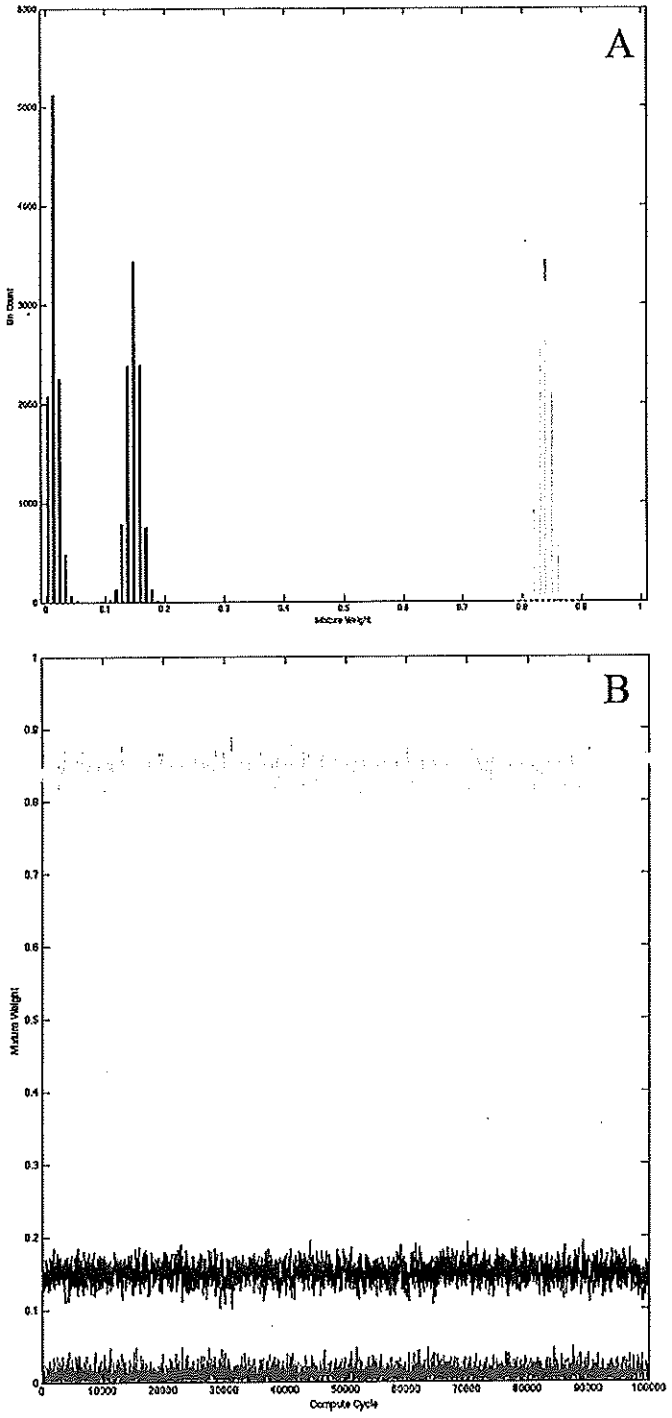

FIG. 2—*Markov chain and histogram of a poor analysis of the complex three-person mixture, Mix3_6. (Panel a) A nonideal histogram (the standard deviation is too small given the complexity of the mixture) of the derived mixture. (Panel b) The corresponding nonideal Markov chain history of the mixture weight sampling.*

The Use of Assumed Known Profiles

The use of assumed knowns was explored by analyzing seven different three-person mixture samples with TrueAllele® Casework and selecting one of the donor samples as an assumed known. Both the correct (assumed known was a donor to the mixture) and incorrect (assumed known was not a donor to the mixture) selection of an assumed known was tested. The match statistics produced when compared with eleven different reference profiles, of which three were the true donors in each mixture, were compared when no assumed known and when an assumed known was used.

Results and Discussion

Allelic Dropout, Locus Dropout, and Peak Imbalance: Single-source Samples

Eight degraded DNA samples were analyzed using TrueAllele® Casework (TA) and compared with their respective reference profiles for generation of the match statistic. Generally, there was a good correlation between the number of alleles observed both above and below the limit of detection (LOD) and the strength of the match statistic (Fig. 4, only S11 samples shown). The LOD values presented pertain solely to GMID as described in Materials and Methods. TA does not apply a LOD value, but instead samples electropherogram signal down to a selected value which was 10 rfu for analyses reported in this study. Baseline noise and peak uncertainty, which is proportional to peak height, among other variables are considered when the data are modeled (9,18,20). However, two samples provided negative log(LR) values when compared to their respective reference profiles (S11 UV and S3 80°C, S3 data not shown). Sample S11 subjected to 80°C produced a positive match statistic yet it displayed fewer alleles above and below the LOD than the sample S11 subjected to sunlight (referred to as UV exposed), which produced a negative match statistic. Thus, further investigation was necessary to determine the cause of such disparate match statistics.

Figure 5 displays electropherograms of the S11 samples incubated at 80°C and UV exposed at room temperature (RT). Six loci of S11 exposed to UV (Panel b) displayed allelic dropout (one allele of the heterozygous allele pair was not visible). Of these six, two loci showed the single visible sister allele below the LOD and unlabeled. The probability values ("p") generated by the TrueAllele® Casework analysis for the true heterozygous genotypes were all extremely low values, thus driving the overall match statistic lower than if neither allele of the heterozygote were present. However, TrueAllele® Casework was able to utilize allele data below the LOD, but distinguishable from baseline noise. An example of this is shown in Fig. 5, Panel (b) where an arrow points to two peaks at D21S11 that are imbalanced and below the LOD. The probability value for the 30,32.2 genotype at D21S11 was estimated at 0.8057. Another example is at the D7S820 locus in Panel (b) where both the 8 and 9 alleles are below the LOD, but TrueAllele® Casework assessed the probability of that genotype at 0.8878, thus demonstrating that TrueAllele® Casework was able to utilize more of the data than is currently available using a traditional threshold based approach. Conversely, the S11 sample subjected to 80°C (Panel a) did not display allelic dropout; instead, it displayed total locus dropout at multiple loci. The log(LR) match statistic (shown in the upper right hand corner of each panel) was significantly higher for S11 subjected to 80°C than subjected to UV even though fewer alleles were visible in the 80°C sample. This difference can be explained by the effect that false homozygotes had on the probability values for the heterozygote genotypes in the UV-treated sample.

Ten amplifications of two different samples (S9 and S1) using genomic template quantities in the stochastic range (30 pg and 10 pg) were analyzed using TrueAllele® Casework and

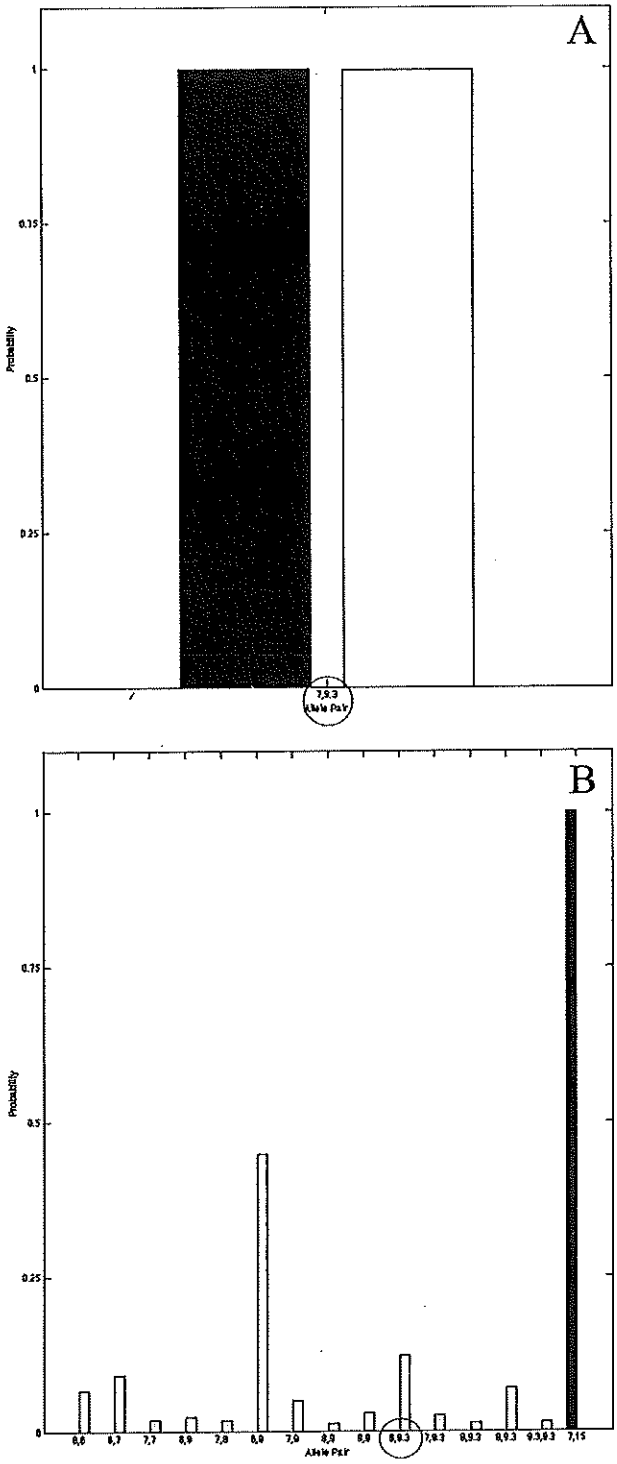

FIG. 3—*Genotype concordance at the TH01 locus for the complex three-person mixture, Mix3_6. (Panel A) Excellent and correct genotype concordance is observed between the predominant contributor for both the ideal and nonideal (poor) analyses shown in Figs 1 and 2. The dark blue column is the derived contributor genotype probability for the nonideal analysis, and the light blue column is the genotype probability derived by the ideal analysis. (Panel B) Poor genotype concordance is observed for the most minor contributor of the ideal and poor analyses. The dark blue column represents the derived contributor genotype probability for the nonideal analysis. It displays a 100% probability for a genotype that is not concordant with the minor contributor (true genotype is 6,9.3). The light blue columns show the derived contributor genotype distribution (due to uncertainty) for the most minor contributor of the ideal analysis. The correct genotypes for the most predominant and most minor contributors are circled (7,9.3 and 6,9.3, respectively).*

compared both to the donor reference profile and a nondonor reference profile for generation of the match statistic. A positive log(LR) was obtained when compared with the corresponding reference donor profile for all 30 pg samples tested, but negative log(LRs) were obtained for three of the five 10 pg samples (data not shown). An inspection of the electropherogram data for one of those 10 pg samples demonstrated the same phenomenon occurred as was described for the degraded samples: false homozygotes, due to allelic dropout, caused a dramatic reduction in the probability value down to zero for a heterozygote allele pair at those loci (data not shown).

Mock Casework Mixture Samples: Two-person Mixtures

Two-person mixture samples were utilized to evaluate how well TA includes the true donors to the mixtures (the sensitivity) and excludes nondonors (the specificity). Mixture samples were chosen purposefully to define the limits of the TA system. The contributor proportions varied from equal to a very tiny (less than 10%) contribution of the minor contributor. All metrics for the TA analyses were considered as described in Materials and Methods. Only the TA analyses that were deemed ideal or acceptable were utilized to assess genotype concordance between independent runs.

Eighteen two-person mixture samples (Mix2_1–Mix2_18) were analyzed with TrueAllele® Casework and interrogated using 11 references profiles. The derived contributors from the mixture profiles were compared to the true donor references and nine nondonor reference profiles. The quality of the TrueAllele® Casework analysis results was evaluated using the metrics as described in Table 1 and Materials and Methods. One requirement of the TrueAllele® Casework review process is to assess the reproducibility; thus, results were compared between two or more independent analyses of the same mixture that were deemed acceptable: Deconvolved mixture weights for the derived contributors were compared to ascertain whether or not they were similar in value, and genotype concordance for both contributors was assessed between analyses. A detailed description of the concordance for all two-person mixtures can be viewed in File S2.

The analyses of all of the eighteen two-person mixtures produced at least two ideal analyses. All analyses provided good or good/fair genotype concordance between the major contributors. Thirteen of the two-person mixture samples provided good or good/fair genotype concordance for the minor contributor. The minor contributor proportion of the mixture for the majority of those samples was greater than 15%, but less than 30%, so a clear distinction between the major and the minor contributors was possible. These samples also showed concordance for the other metrics, such as mixture weights and the log(LRs).

Five samples provided a fair or fair/poor genotype concordance for the minor contributor and for these samples, and the minor contributor proportion was less than 15%. Three of the five samples (Mix2_1, Mix2_3, and Mix2_7) failed to provide reproducible log(LR)'s for the minor contributor. However, Mix2_3 and Mix2_7 did provide consistent mixture weights for both the minor and major contributors. Mix2_1 failed to yield a positive log(LR) for the minor contributor; however, upon examination of the electropherogram, only two small alleles at D3S1358 and TH01 (144 rfu and 92 rfu, respectively) were observed that were solely attributable to the minor contributor (Fig. 6), and thus, the negative log(LR) appears to be appropriate.

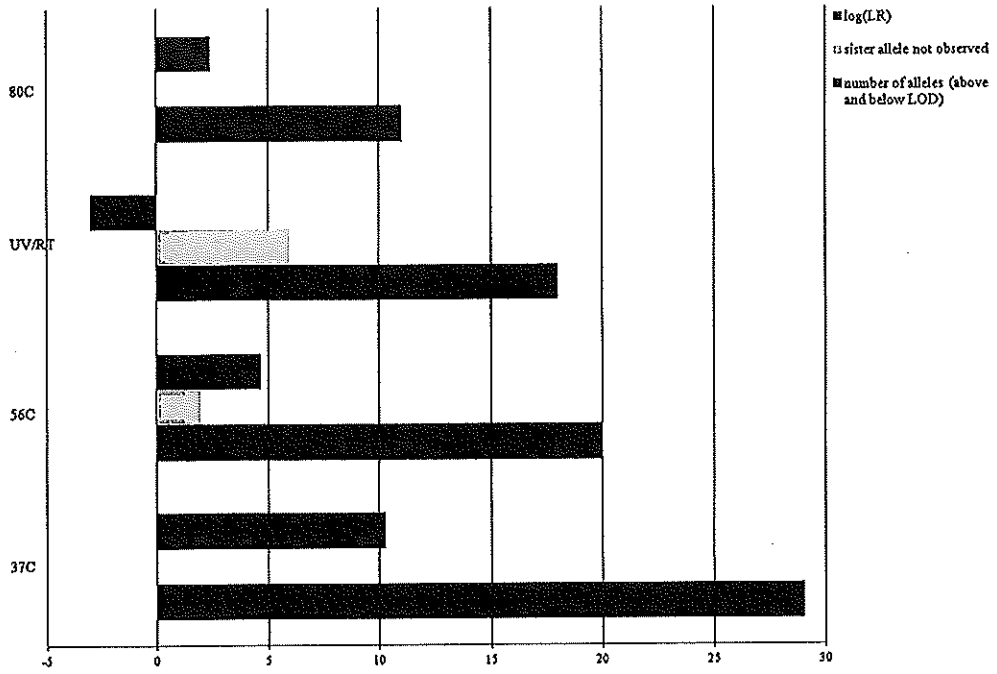

FIG. 4—*Relationship between the number of alleles above and below the LOD, the sister allele not observed and the log(LR) (match statistic). Only sample S11 is shown, and all analyses depicted were performed for 100K cycles. The maximum log(LR) for S11 (match probability) is 19.4927. All samples were incubated for 3 months. Key: 37C, 56C, and 80C = temperature incubated in degrees centigrade, UV = ultra violet light exposure at room temperature (RT).*

It should be noted that the three samples lacking good log (LR) reproducibility for the minor contributor had one analysis performed at 25K and the other at 100K. Thus, additional runs at 50K or more may be merited to produce more consistent match statistics for the minor contributor. The two-person mixture samples with a low-level minor contributor were deconvolved with great accuracy in that no nondonors were falsely included. The minor contributors displayed more genotype uncertainty, as would be expected with such low-level proportions.

Mixture Weight Accuracy

The accuracy with which TrueAllele® Casework deconvolutes mixture weights for two-person mixtures was assessed. Figure 7 displays a comparison between the targeted mixture weights of 17 mixture samples based upon the quantitation data, the estimated mixture weights assessed by manual calculation and the TrueAllele® Casework deconvolved mixture weight derivations. An inspection of the graph reveals that the manual and TrueAllele Casework derived mixture weights were extremely similar, but somewhat different from the targeted mixture weights based upon the DNA quantitation data.

No manual calculation was performed for the Mix2_5 sample as no clear minor contributor could be identified. The TrueAllele® Casework mixture weight value for the minor contributor was far from the targeted mixture weight for Mix2_5 (49% vs. 20%, respectively), but a review of the electropherogram data demonstrates that the TrueAllele® Casework derived mixture weight was more accurate as it is clear that the mixture was very close to a 1:1 combination of the two components (Fig. 8). The Mix2_8 and Mix2_9 samples were dehydrated and not re-quantitated, so the DNA concentrations were unknown.

Three-person Mixture Samples

Fifteen three-person mixture samples (Mix3_1–Mix3_15) were analyzed with TrueAllele® Casework and interrogated using 11 reference profiles; however, only ten of these mixtures were assessed for genotype concordance and reproducibility. The other five mixture samples were not repeatedly analyzed and thus were utilized solely for the specificity test. Detailed information about the samples can be found in File S1 and detailed assessments of the TA analysis for each sample can be viewed in File S3. The reference profile population contained the three donors for each of the mixtures in addition to eight nondonors. The quality of the TrueAllele® Casework analysis results was evaluated using the metrics as described in Materials and Methods.

The three-person mixtures present a far more complex analysis for either a human or the TrueAllele® Casework process. The three-person mixtures utilized were challenging and purposefully chosen for this study to assess the limitations of the TrueAllele® Casework process. Given the complexity of the mixture samples, the 25K cycle number was abandoned and those analyses are not included in the File S3. In general, when all of the metrics provided values within the desired ranges, for example, Mix3_4 (50K, 100K, and 100K2X runs), the concordance observed between runs was very good. Analyses that showed examples of the convergence value exceeding 1.2 were observed for all cycle numbers employed (50K, 100K, and 200K). This may indicate that longer sampling (more cycles) might be merited or it may be that the challenging nature of the mixture makes it recalcitrant to an ideal resolution, even at a very high cycle number. While convergence values below 1.2 are ideal, many examples of concordant runs were observed with higher than ideal convergence values.

Mix3_10 proved to be a challenging sample. Seven independent analyses were initially performed, consisting of five 100K

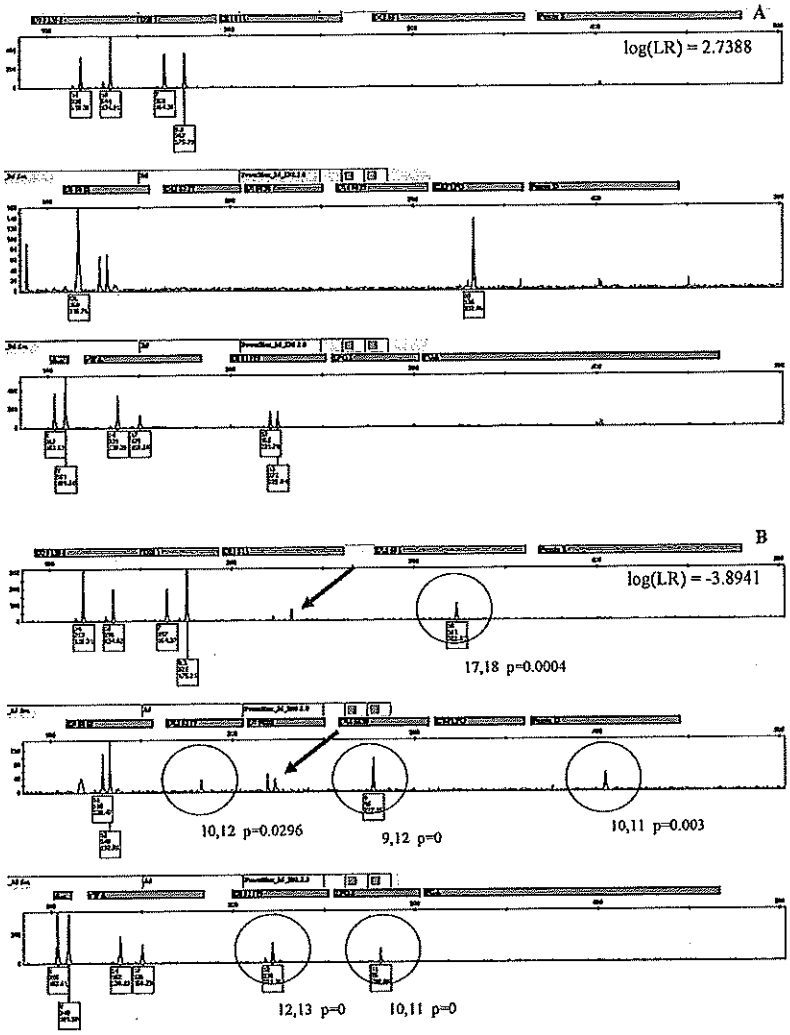

FIG. 5—*PowerPlex® 16 System typing data for sample S11 incubated at 80°C (Panel A) and for sample S11 incubated at RT with UV exposure (Panel B). Circled peaks indicate loci where the sister allele is missing. The correct, heterozygous genotype is indicated below and to the right of the peaks. Probability values ("p") for the true genotypes are adjacent to the genotypes. An arrow points to two peaks at the D21S11 locus that have poor allele balance (39%) and are both below the LOD. An arrow points to D7S820 where both peaks are below the LOD. The match statistic is displayed in the upper right-hand corner of panels (A) and (B).*

and two 200K runs. Only one of those seven provided an ideal analysis. Upon re-inspection of the electropherogram data, it was noted that a large spike at a size of approximately 399 bases was evident (data not shown). The allele calls associated with that spike were removed using the Request module of TrueAllele® Casework and the sample re-analyzed at 100K two times and once at 200K ("edited" appears in the name of the follow-up analyses, File S3). One of the 100K analyses and the 200K analysis provided concordant results. It was noted that the two concordant runs with the spike removed provided larger match statistics for the three contributors than the single ideal analysis that included the spike. This result is consistent with an increase in genotype certainty once the spike was removed.

In nine of the ten three-person mixtures, all nondonors for every ideal or acceptable and even poor analysis were excluded (consistently provided negative log(LR) match statistics). Mix3_6 did display a small positive match statistic for a noncontributor (S6; 3.057 times more likely {log(LR) 0.485}) for the under-sampled 50K analysis; however, this was rated a poor analysis prior to comparison with any reference samples and more importantly, this positive match statistic for comparison to S6 was not reproducible. The two ideal analyses provided log (LRs) of −1.0538 and −1.0291, reproducibly providing no statistical support for inclusion of the nondonor, S6 (data not shown). An examination of the electropherogram data demonstrates the selectivity of the TrueAllele® Casework analysis process as nearly every allele of reference S6 is shared with the Mix3_6 mixture profile (Fig. 9), yet no statistical support was generated for reference S6 as a contributor to the mixture.

Three-person Mixtures with an Assumed Known

The use of an assumed known for three-person mixtures was explored with respect to its effect on the TrueAllele® Casework analysis process. Assumed knowns are frequently utilized in forensic DNA analysis and mixture de-convolution as some samples, such as intimate ones, might reasonably be expected to contain DNA from the source of the sample (e.g., a vaginal swab would be expected to contain victim DNA). To demonstrate this effect, an assumed known was designated for one of the true donors for each of the seven-three-person mixtures selected for this demonstration. A minor contributing donor was chosen for

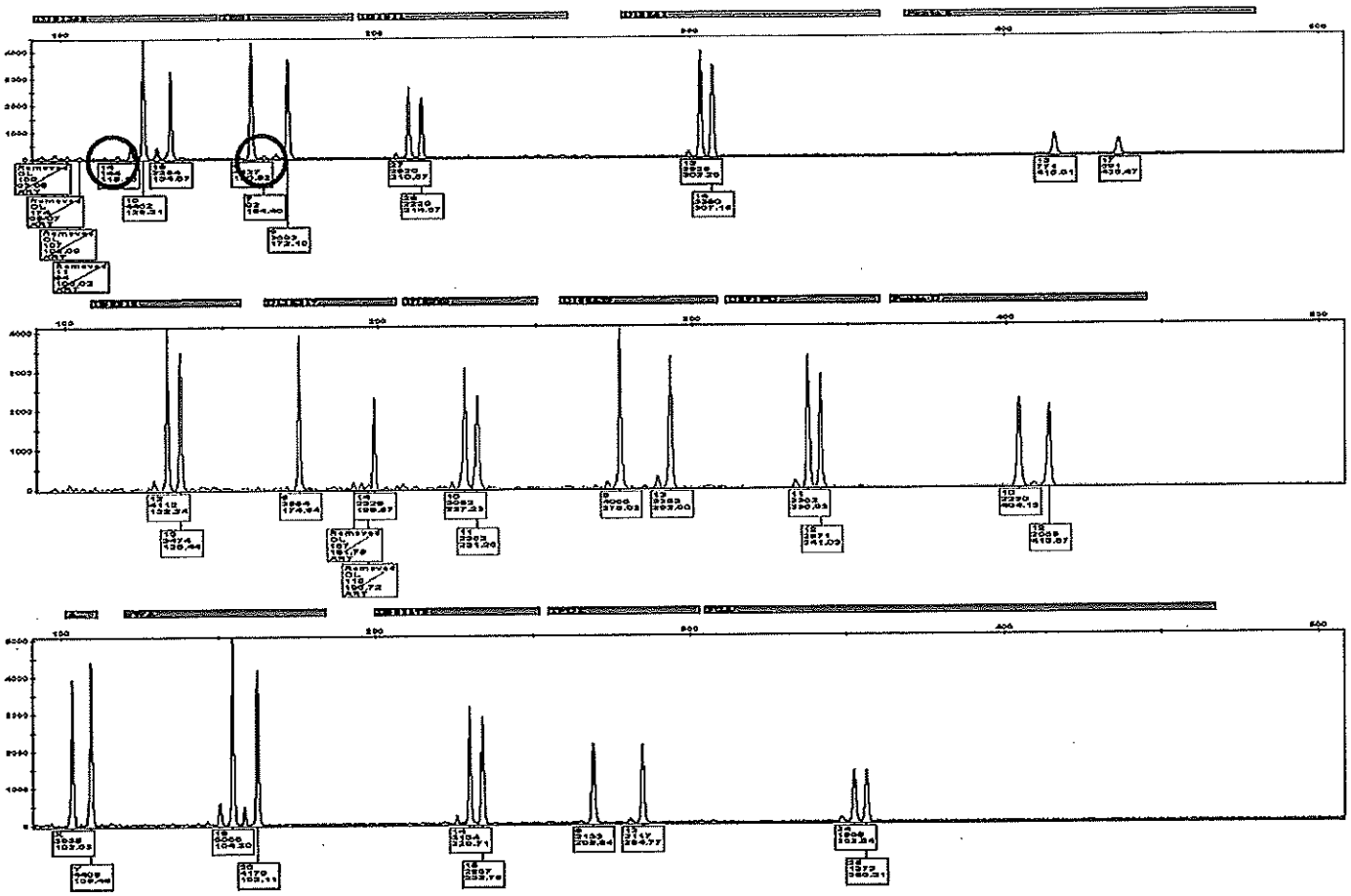

FIG. 6—*PowerPlex® 16 System profile of the Mix2_1 sample. Obligate alleles to the minor contributor are circled (14 at D3S1358 and 7 at TH01).*

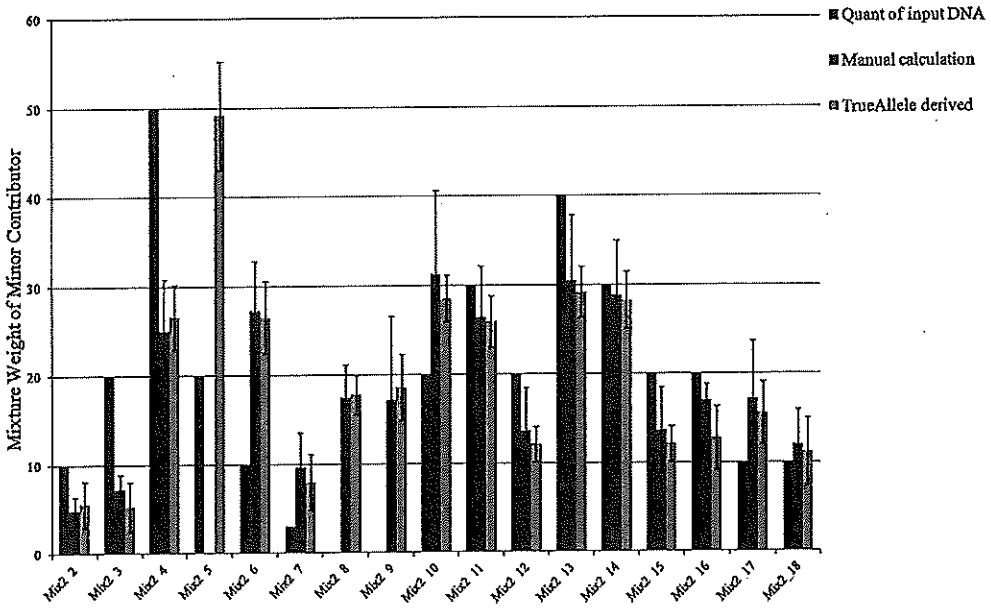

FIG. 7—*Accuracy of mixture weight assessment by TrueAllele® Casework for the minor contributor of two-person mixture samples. The "n" ranged from 2 to 9, with the average being 6.4 loci for manual mixture weight estimates.*

designation as an assumed known except for mixtures Mix3_3 and Mix3_4, where the predominant donor was designated. Table 2 provides examples of the use of a correct (individual was a donor to the mixture) and incorrect (individual was not a donor to the mixture) assignment of an assumed known. Mix3_4 and Mix3_8 were tested using assumed knowns that were actual

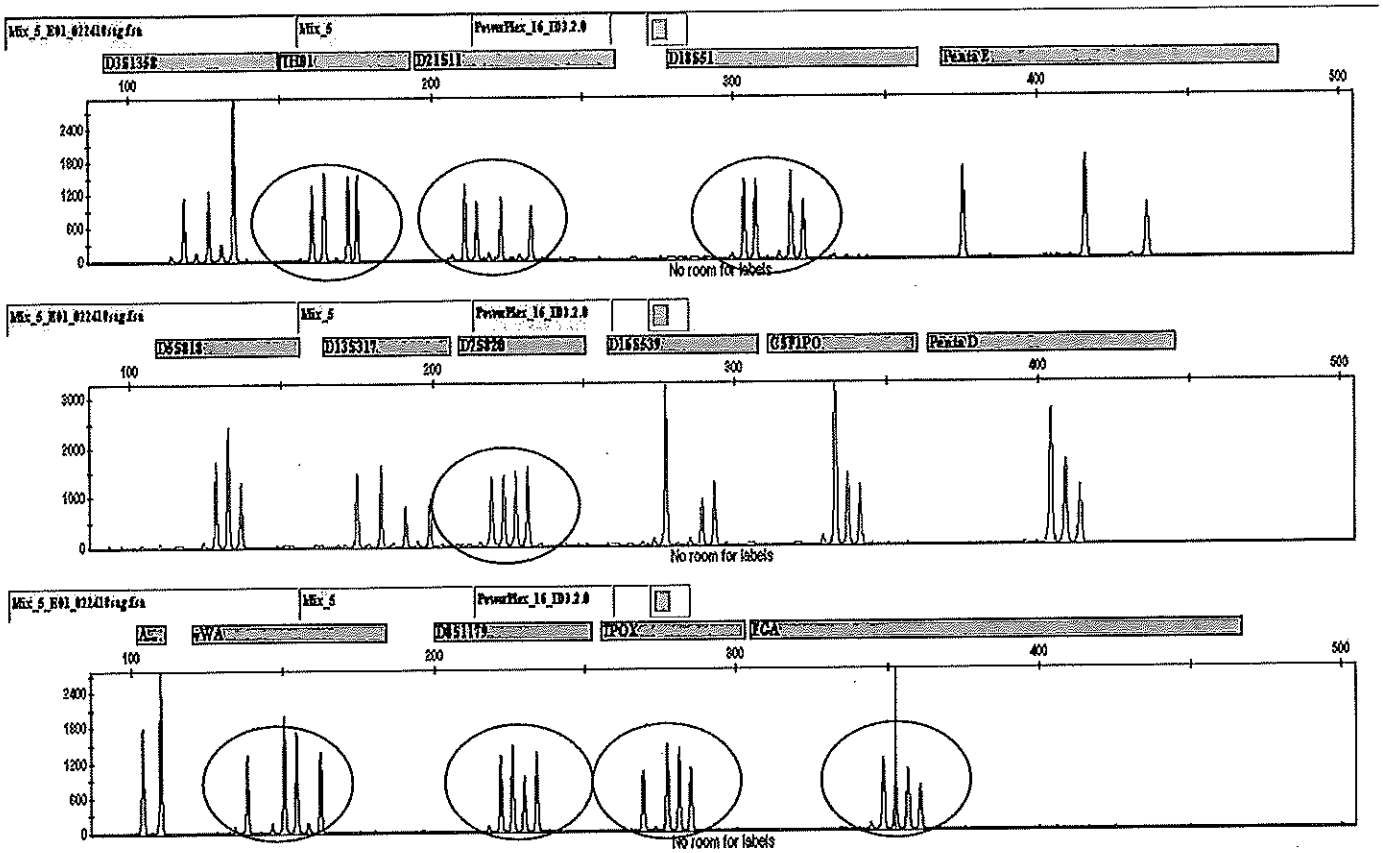

FIG. 8—*PowerPlex® 16 System profile of the Mix2_3 two-person mixture. Loci displaying four alleles nearly equal in height representing the 50:50 mixture are circled.*

donors and assumed knowns that were not donors to the mixtures. The log(LR) for an assumed known is the maximum value for that profile and is shown in bold.

In general, the use of a correct assumed known increased the match statistic for the remaining contributors and can increase the KL value (the information content of the derived contributors); however, for the Mix3_4, Mix3_6, and Mix3_7 samples, the log (LR) was only slightly changed. The impact of an assumed known is greater when teasing apart contributors similar in mixture weight or when applied to a minor contributor (pers. obs.). Deconvolved contributor genotypes with little to no uncertainty typical of a major contributor demonstrate only marginal gain in match score as a result of assigning an assumed known.

The question of whether or not the software could be confused by an operator error when assigned an assumed known was addressed by choosing a nondonor as an assumed known. An example of this is shown for samples Mix3_4 and Mix3_8. The impact on the log(LR) for the remaining minor contributors was a reduction in the value, but value for the predominant contributor was relatively unaffected. The use of an incorrect assumed known did not result in the inclusion of noncontributors to the mixtures among the eleven reference profiles tested (data not shown) nor in the exclusion of true donors.

Four-person Mixture Samples

Seven four-person mixtures (Mix4_1–Mix4_7) were analyzed using the TrueAllele® Casework system. Supplementary File 4 provides a detailed summary of the results. Although the 25K cycle number analysis was initially performed for these complex four contributor mixture profiles, 25K cycles were deemed insufficient and those analyses are not included in File S4. As with the three-person mixtures, the four-person mixtures required multiple analyses to produce reproducible and concordant results. Mix4_3 was a very challenging sample and eight independent analyses were performed generating four ideal or acceptable analyses. The concordance between the 100K2X, 200K, 200K3X, and 200K4X analyses was good except for the match statistic produced for the nondonor, S4, which fluctuated around zero giving small negative (−0.0536 and −0.0104, 200K and 200K4X, respectively) and small positive (0.686 and 0.0869, 100K2X and 200K3X, respectively) log(LR)'s. An examination of the electropherogram for Mix4_3 demonstrated that as with the three-person mixture (Mix3_6), the nondonor shared nearly every allele with the mixture profile (data not shown). The match statistic for S4 was not reproducible among the four analyses utilized for genotype concordance.

Of the seven samples analyzed, six provided at least two ideal and concordant analyses. The analysis of one sample, Mix4_1, did not produce more than one ideal analysis of the seven performed, so genotype concordance was not assessed. Two samples, Mix4_4 and Mix4_5, provided small yet reproducible negative log(LRs) for the most minor of the minor contributors. An examination of the electropherogram data provided an explanation for this statistical result as the mixture displayed allele drop-out at three or more loci, peaks below the stochastic threshold, masking of alleles, and alleles falling in the stutter position which corresponded to the minor contributor, S8 (data not shown). Given the complexity of the four-person mixture samples, additional analyses would be merited for casework samples

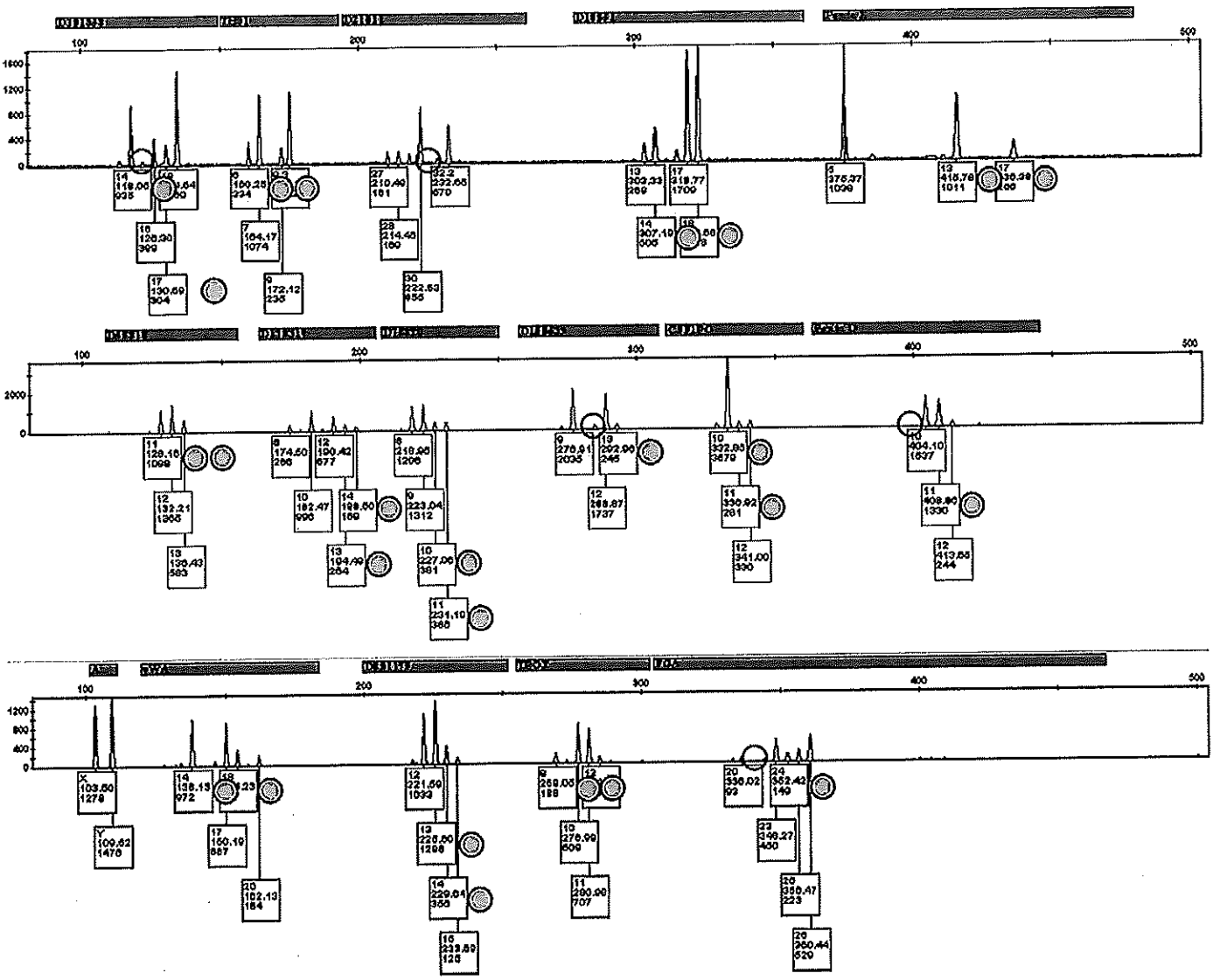

FIG. 9—*Mix3_6 PowerPlex® 16 System profile. The pink dots are adjacent to allele calls that are consistent with sample S6. Two dots indicate that S6 was homozygous for the allele. The black circles at the baseline encircle either a stutter allele consistent with an allele for S6 at that locus, or the position at which S6 would have an allele (i.e., no peak was observed in the Mix3_6 mixture profile).*

of similar intricacy; however, further testing was not conducted given that four contributor mixtures are not routinely interpreted at VDFS (18,19).

Specificity of Differentiating Relatives

The ability of the TrueAllele® Casework system to differentiate between closely related people was tested. First-degree relatives ("sons") were manually synthesized for seven reference profiles. Six of the reference profiles for which "sons" were created were donors to the mixture samples. Thus, it would not be unexpected to observe small positive match statistics for such close relatives. Only ideal or acceptable analyses of the two-, three-, and four-person mixtures were utilized for this test. The match statistic for all synthetic relatives was negative for the two-person mixtures (data not shown). Table 3 displays the log (LR) values generated for three- and four-person mixture analyses which produced a positive match statistic when compared with the "sons".

The analysis of three of the three-person and one of the four-person mixture samples produced derived contributors that resulted in positive log(LRs) when compared to a synthetic son of a donor to the mixture. Mix3_7 and Mix3_8 displayed reproducible small positive match statistics for a "son" of S1. While, the match statistic for the "son" of S1 was significantly lower than the match statistic for S1 in Mix3_8, it was approximately the same as S1 in Mix3_7. Reference S1 is the most minor contributor in both mixtures and exhibited allelic dropout at several loci (data not shown). There was also a nonreproducible small log(LR) produced for a "son" of S8 in Mix3_10-ed. The analysis of one-four-person mixture sample, Mix4_4, provided a positive match statistic for a "son" of a donor to the mixture. The positive log(LR) for the "son" was very small (1.002–1.79 times more likely). It is interesting to note that the donor (father of the son) was the most minor contributor to Mix4_4 and a reproducible negative log(LR) was produced by the analysis of the sample.

When synthetic "brothers" of contributors to the same two-, three-, and four-person mixture samples were compared, only one sample, three-person mixture Mix3_2, displayed a positive match statistic for the "brother". Only one analysis of Mix3_2 (analyzed at 100K2X) displayed a small positive match score of 1.0659 when the comparison to the synthetic brother of one of

TABLE 2—*The effect of an assumed known on the match statistic for three contributor mixtures. All samples were analyzed at 100K cycles. The assumed knowns used for Mix3_3 and Mix3_4 were the most predominant contributors, whereas a minor contributor was utilized for all others. The match statistic for the assumed known (maximum value) is shown in bold. The corresponding match statistic for the same donor when not selected as an assumed known in a different analysis is italicized. The match statistics for all three contributors are listed in order (e.g., S1, S5, S7) for both analyses (no assumed known and with an assumed known).*

Mixture Sample	Assumed Known	AK Donor to Mixture?	log(LR)
Mix3_1	No	–	*8.08*, 10.41, 19.45
	Yes	Yes	**16.33**, 13.21, 19.49
Mix3_2	No	–	−0.75, *11.01*, 12.13
	Yes	Yes	3.18, **20.96**, 15.85
Mix3_3	No	–	5.43, 10.73, *14.63*
	Yes	Yes	10.47, 11.23, **19.49**
Mix3_4	No	–	6.42, 5.50, *19.49*
	Yes	Yes	6.41, 5.96, **19.49**
	Yes	No	4.6, 1.23, *19.49*
Mix3_6	No	–	2.93, *11.85*, 19.47
	Yes	Yes	2.58, **20.96**, 19.49
Mix3_7	No	–	0.67, *3.65*, 18.59
	Yes	Yes	1.44, **21.96**, 18.6
Mix3_8	No	–	4.13, *6.13*, 17.57
	Yes	Yes	8.39, **21.96**, 18.25
	Yes	No	2.03, *5.94*, 17.69

AK, assumed known.

the donors was performed (data not shown). This was not reproducible.

Specificity

The specificity of the TrueAllele® Casework analysis process was more thoroughly addressed using 100 synthetic PowerPlex® 16 reference profiles, kindly provided by Cybergenetics, to compare with the derived contributors genotypes of two-, three-, and four-person mixtures. Multiple analyses of eighteen two person, fourteen three-person and seven-four-person deconvolved mixture samples were utilized. Only ideal or acceptable TrueAllele® Casework analyses were utilized. A total of 21,400 comparisons were performed for the derived contributors. No positive log(LR)s were produced for the comparisons performed for the two- and three-person derived contributors (data not shown). Of all of the derived contributors (214) for all of the analyses performed of the 39 total samples analyzed, only one provided a small (2.9 times more likely) and nonreproducible match statistic. Results for the most common match scores for the four-person mixtures are displayed in Fig. 10. The results of this test indicate that the TrueAllele® Casework analysis process is highly specific, even for complex three- and four-person mixtures.

Conclusion

The TrueAllele® Casework system accurately inferred problematic single-source sample profiles and generated positive match statistics. Generally, the greater the number of loci with alleles above the limit of detection, the more discriminating the match statistic; however, exceptions were observed if the single-source profile contained multiple false homozygotes. In those instances, a negative match statistic was observed due to a very low or zero probability being generated for the true heterozygote genotype at those loci. TrueAllele® Casework analysis was demonstrated to take advantage of additional information not utilized in a traditional threshold based analysis, such as alleles below the LOD and assigned probability values greater than zero to the correct genotypes.

Two-person mixture samples were easily resolved with the TrueAllele® Casework system with great specificity and discriminating match statistics unless the minor contributor was less than a 10% contributor to the mixture. When the minor contributor provided only a very small proportion of the DNA in the mixture, the match statistic reflected that weak contribution with

TABLE 3—*Three and four-person mixture samples which provided positive match scores for synthetic sons. Dark gray fill and bolded number indicates the match statistic for the donor included in the mixture (highest value generated for the comparison with the reference sample). Light gray fill and italicized number indicates a positive match statistic for a "son" of a donor to the mixture. S1–S11 refers to sample name. Not shown are the results for comparisons to "brothers" of the donors to the mixtures.*

	S1	S1_son	S4	S4_son	S5	S5_son	S6	S8	S8_son	S10
3 person mixtures										
Mix3_7 100K2X	**0.668**	−0.150	−9.323	−9.015	−5.840	−6.054	−9.515	3.387	−3.195	−8.424
Mix3_7 100K2X	−24.490	−26.157	−23.057	−25.163	**18.590**	−26.413	−26.890	−23.428	−26.870	−26.725
Mix3_7 100K2X	0.468	*1.342*	−9.659	−8.628	−5.851	−5.950	−10.009	**3.647**	−2.853	−6.734
Mix3_7 200K	**1.404**	*0.791*	−9.070	−8.012	−5.728	−5.712	−10.560	**3.672**	−2.743	−8.496
Mix3_7 200K	0.945	0.140	−9.410	−8.636	−5.775	−5.330	−9.748	3.605	−2.641	−8.203
Mix3_7 200K	−24.490	−26.188	−23.717	−25.835	**18.588**	−27.073	−26.565	−23.299	−26.878	−26.380
Mix3_8 100K2X	**4.129**	*0.234*	−10.648	−8.149	−3.554	−5.224	−7.045	**6.126**	−0.338	−6.790
Mix3_8 100K2X	−21.214	−21.625	−21.935	−17.852	**17.575**	−22.281	−25.652	−18.319	−21.712	−24.039
Mix3_8 100K2X	3.090	0.199	−8.567	−5.418	−4.499	−4.756	−6.088	5.033	−0.773	−7.022
Mix3_8 100K3X	3.341	0.182	−10.585	−7.533	−2.875	−5.385	−7.209	5.975	−0.538	−6.687
Mix3_8 100K2X	**3.864**	*0.818*	−11.299	−7.934	−0.678	−4.045	−8.987	**6.399**	−0.840	−7.254
Mix3_8 100K2X	−20.640	−18.904	−21.404	−16.901	**17.346**	−20.465	−25.347	−15.784	−19.532	−21.679
Mix3_10-ed 100K	0.906	−3.998	−18.764	−8.834	4.753	−5.347	−12.771	9.551	−0.223	−9.649
Mix3_10-ed 100K	−6.701	−8.054	−23.744	−13.919	**6.636**	−11.562	−19.780	**10.552**	−4.562	−11.529
Mix3_10-ed 100K	**5.904**	−1.487	−15.747	−9.026	2.377	−3.790	−10.635	6.174	*0.936*	−8.448
4 person mixtures										
Mix4_4 100K2X	−24.400	−24.337	−30	−30	−27.073	−27.073	−19.002	−27.145	−20.593	**17.159**
Mix4_4 100K2X	−8.983	−7.388	1.585	−6.232	−8.339	−5.727	2.539	−1.886	−0.111	−4.441
Mix4_4 100K2X	−8.285	−6.954	1.453	−6.981	−6.680	−6.364	2.859	−2.674	*0.001*	−4.439
Mix4_4 100K2X	−10.065	−7.549	**1.705**	−6.665	−7.805	−5.116	**3.099**	**1.735**	−0.101	−4.475
Mix4_4 100K4X	−24.297	−24.337	−30	−30	−27.073	−27.073	−21.493	−27.145	−20.490	**17.262**
Mix4_4 100K4X	−8.128	−7.071	**1.446**	−7.189	−7.101	−5.990	2.530	−2.511	0.124	−4.550
Mix4_4 100K4X	−8.005	−6.789	1.376	−6.610	−8.198	−5.380	2.535	−1.998	−0.135	−5.003
Mix4_4 100K4X	−8.390	−7.476	1.314	−6.363	−7.079	−4.944	**3.170**	**1.680**	*0.252*	−4.383

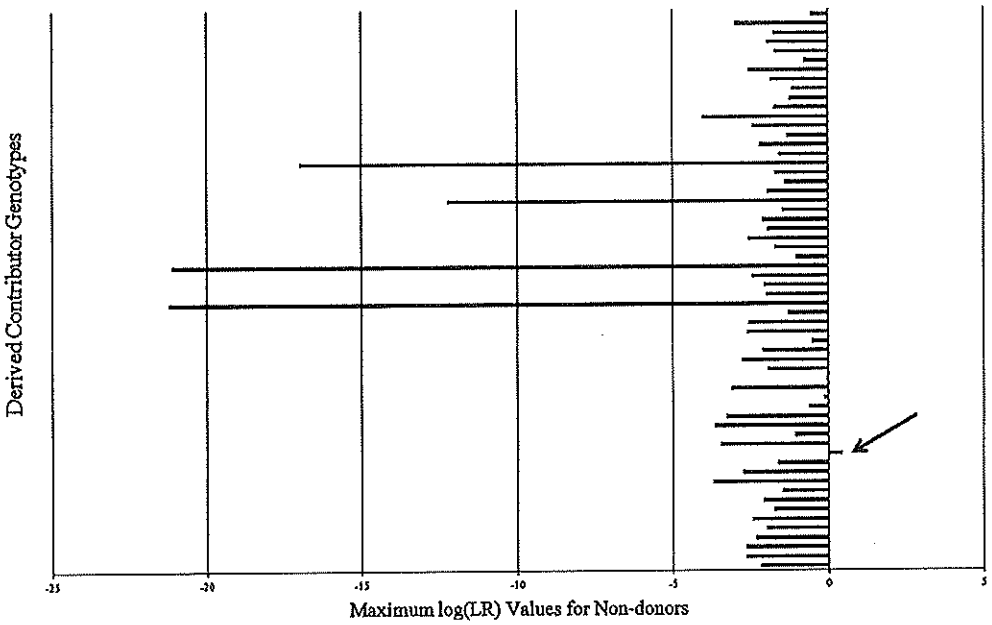

Fig. 10—*Maximum log(LR) values obtained by comparison of the derived contributors for the four-person mixture samples to 100 synthetic profiles (non-donors). Each bar represents a derived contributor of the four-person mixture samples. Only one derived contributor for mixture Mix4_3 (arrow) provided a positive log(LR).*

uncertainty resulting in lower match statistics. Of the eighteen samples, only Mix2_1 provided a negative match statistic for the minor contributor. An inspection of the electropherogram demonstrated that only two very small alleles were solely attributable to the minor contributor. All nondonors to the mixtures were definitively excluded (generated negative log(LR) values).

TrueAllele® Casework accurately assessed the mixture weights for two-person mixtures. When compared with the estimated mixture weights based upon DNA quantitation and template input quantities, TrueAllele® Casework provided a more accurate estimate based on an evaluation of the electropherogram data, comparable to the manually measured values calculated using peak heights.

Three- and four-person mixtures greatly increased the complexity and the genotype uncertainty of the analysis which was reflected in the match statistics for the minor contributors. For the 10 three-person mixture samples repeatedly analyzed using the TrueAllele® Casework process, none of the runs used for concordance provided a positive match statistic for a nondonor to the mixture. One sample (Mix3_6) provided a small (3.057 times more likely) match statistic for a nondonor, but it was not reproducible and only observed in a nonideal analysis (50K); thus, it could safely be excluded when drawing conclusions based on ideal runs for that sample. An inspection of the electropherogram demonstrated that the aforementioned nondonor shared nearly every allele with the mixture profile; therefore, the successful exclusion of the nondonor provides evidence supporting the specificity of the TrueAllele® Casework analysis process. One sample, Mix3_10, appeared recalcitrant to obtaining reproducible analyses. However, upon re-inspection of the electropherogram data, a large polymer spike was evident and once the allele information associated with that spike was deleted, additional ideal analyses were obtained with higher match statistics for the contributors, reflecting an increase in genotype certainty once the spike was removed.

Of the seven-four-person mixture samples repeatedly analyzed by the TrueAllele® Casework process, only one sample, Mix4_3, provided small, but nonreproducible positive match statistics for a nondonor. Ideal analyses at 100K and 200K (100K2X, 200K, 200K3X, and 200K4X) provided both positive and negative log(LRs) hovering around an uninformative log (LR) of zero for the nondonor. As with the Mix3_6 three-person mixture discussed above, the nondonor shared nearly every allele at all loci with the mixture profile and thus not unexpectedly, provided a difficult challenge for the TrueAllele® Casework analysis process. Analysis of two of the four-person mixture samples, Mix4_4 and Mix4_5, produced small, but reproducible negative log(LRs) for the most minor contributor. An inspection of the electropherogram data provided a reason for these exclusions as the donor displayed allelic dropout at multiple loci, masking of alleles, alleles in the stutter position, and alleles below the stochastic threshold. This demonstrates that TrueAllele® Casework analysis process requires sufficient evidential support for a true donor to derive a positive match statistic.

The use of an assumed known was explored with respect to its effect on the TrueAllele® Casework analysis process. Generally, the use of a correct assumed known, especially for a minor contributor, increased the match statistic for the remaining contributors by one or more bans and strengthened the KL value for the derived contributors; however, for some samples, the match statistic remained little altered. The use of an incorrect assumed known did reduce the match statistic for the true donors; however, it did not result in the inclusion of nondonors to the mixtures among the eleven reference profiles tested. Only a small study was conducted as it is unlikely that a nondonor would be selected as an assumed known.

Two person, three-person and four-person mixture runs were used to assess the ability of the TrueAllele® Casework system to differentiate between closely related people. First-degree relatives ("sons") were successfully excluded for all 35 mixture samples tested except for three-three-person and one-four-person mixtures. The positive match statistic for the "son" of a donor for two of the three-person mixtures was reproducible and small. The third example of a positive match statistic for a "son" of a donor to a three-person mixture was not reproducible. One

four-person mixture sample (Mix4_4) provided a positive match statistic for a "son" of a donor to the mixture; however, the LR was not reproducible and was small. When synthetic "brothers" were compared with the same two-, three-, and four-person mixture samples analyzed by the TrueAllele® Casework system, only a single analysis of one sample (Mix3_2), a three-person mixture, provided a small positive match statistic when compared to a "brother" of one of the donors to the mixture. That result was not reproducible. The potential for rendering a relatively small positive match statistic for a first degree relative of a contributor to a complex mixture is to be expected.

The specificity of the TrueAllele® Casework analysis process was tested using 100 synthetic PowerPlex® 16 reference profiles which were compared to the derived contributors of two-, three-, and four-person mixtures. Of the 214 derived contributors from the analyses performed, 21,400 comparisons were completed. Only one provided a very small and nonreproducible match statistic, indicating that the TrueAllele® Casework analysis process is highly specific, even for complex three- and four-person mixtures.

The TrueAllele® Casework process has been demonstrated to be sensitive and specific in its ability to include true donors and exclude or find no statistical support for nondonors. STR data displaying a great deal of allelic dropout and false homozygotes may produce a negative match statistic when compared to a true donor. This typically reflects the weakness of the profile and the conservativeness of the TrueAllele® Casework process.

Based upon this body of work and extensive training, the VDFS has implemented the use of TA in selected cases beginning in 2014. An inconclusive log(LR) range of ±1 log unit (ban) has been established for casework analysis based on the specificity studies. Interestingly, a small number of cases where the human review of a mixture sample resulted in a finding of inconclusive with regard to the person of interest were also analyzed by TA and the match scores supported that finding (L. Schiermeier-Wood, pers. obs., 26). While the VDFS does not routinely interpret complex mixtures in which there is evidence supporting four or more donors, the analysis of four-person mixtures is not precluded. The studies reported herein demonstrate that even with complex four-person mixtures, TA is capable of performing an accurate, sensitive, and specific analysis.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

File S1. Table listing all of the samples used for testing the TrueAllele® Casework software program except for the synthetically created profiles obtained from Cybergenetics (100 profiles uploaded to the system as text files).

File S2. Two person mixture results.

File S3. Three person mixtures.

File S4. Four person mixtures.

EXHIBIT 7

OPEN ACCESS Freely available online

PLOS ONE

TrueAllele Casework on Virginia DNA Mixture Evidence: Computer and Manual Interpretation in 72 Reported Criminal Cases

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Abstract

Mixtures are a commonly encountered form of biological evidence that contain DNA from two or more contributors. Laboratory analysis of mixtures produces data signals that usually cannot be separated into distinct contributor genotypes. Computer modeling can resolve the genotypes up to probability, reflecting the uncertainty inherent in the data. Human analysts address the problem by simplifying the quantitative data in a threshold process that discards considerable identification information. Elevated stochastic threshold levels potentially discard more information. This study examines three different mixture interpretation methods. In 72 criminal cases, 111 genotype comparisons were made between 92 mixture items and relevant reference samples. TrueAllele computer modeling was done on all the evidence samples, and documented in DNA match reports that were provided as evidence for each case. Threshold-based Combined Probability of Inclusion (CPI) and stochastically modified CPI (mCPI) analyses were performed as well. TrueAllele's identification information in 101 positive matches was used to assess the reliability of its modeling approach. Comparison was made with 81 CPI and 53 mCPI DNA match statistics that were manually derived from the same data. There were statistically significant differences between the DNA interpretation methods. TrueAllele gave an average match statistic of 113 billion, CPI averaged 6.68 million, and mCPI averaged 140. The computer was highly specific, with a false positive rate under 0.005%. The modeling approach was precise, having a factor of two within-group standard deviation. TrueAllele accuracy was indicated by having uniformly distributed match statistics over the data set. The computer could make genotype comparisons that were impossible or impractical using manual methods. TrueAllele computer interpretation of DNA mixture evidence is sensitive, specific, precise, accurate and more informative than manual interpretation alternatives. It can determine DNA match statistics when threshold-based methods cannot. Improved forensic science computation can affect criminal cases by providing reliable scientific evidence.

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Competing Interests: I have read the journal's policy and have the following conflicts. Mark Perlin is a shareholder, officer and employee of Cybergenetics in Pittsburgh, PA, a company that develops genetic technology for computer interpretation of DNA evidence. Cybergenetics manufactures the patented TrueAllele® Casework system, and provides expert testimony about DNA case results. Kiersten Dormer and Jennifer Hornyak are current or former employees of Cybergenetics. Lisa Schiermeier-Wood and Dr. Susan Greenspoon are current employees of the Virginia Department of Forensic Science, a government laboratory that provides expert DNA testimony in criminal cases and is adopting the TrueAllele Casework system. This does not alter our adherence to all the PLOS ONE policies on sharing data and materials.

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Introduction

DNA analysis is the forensic gold standard in human identification [1]. By deriving a genotype from minute amounts of biological material [2], scientists can help identify individuals connected to a crime scene.

With increased societal expectations [3], crime laboratories now process more challenging DNA evidence. Such samples are typically mixtures of two or more individuals, with DNA that may be damaged, degraded or present in small amounts [4]. DNA from one person expresses only one or two alleles at a genetic locus, and so is readily genotyped by visual inspection. Mixture data, however, may present multiple genotype alternatives that complicate interpretation.

Human analysts may simplify short tandem repeat (STR) [5] interpretation by applying a threshold that reduces quantitative data into all-or-none events [6]. This approach works well with single source samples that contain only one genotype. But with mixtures, thresholds discard the quantitative contributions of each genotype, along with the peak height pattern. Threshold-based methods can reduce identification information, render probative data "inconclusive", and potentially infer an incorrect genotype [7].

An "analytical" threshold helps human analysts distinguish between allelic data peaks and baseline instrument noise. The Combined Probability of Inclusion (CPI) mixture interpretation method first applies this analytical threshold to decide which peaks at a locus are sufficiently tall to be considered alleles. If a reference individual's alleles are included in this set of mixture alleles, then CPI uses all the alleles in the mixture set to calculate a match statistic (the inclusion probability) as the square of the sum of the allele frequencies. (Allele determination can be viewed as a separate human interpretation step that precedes the CPI statistical calculation step. For clarity in this paper, we consider

the entire data analysis procedure to comprise the CPI interpretation method). The method does not make assumptions about the number of contributors.

There is naturally occurring random variation in the polymerase chain reaction (PCR) [8]. Therefore, repeat amplifications of the same DNA template quantity will produce varying peak heights. A pair of heterozygote sister alleles may express one taller peak, along with a considerably shorter peak, thereby creating a situation where the heterozygote could be misinterpreted as a homozygote. The analytical threshold does not address this situation [9].

In 2010, the United States Scientific Working Group on DNA Analysis Methods (SWGDAM) published guidelines to help resolve such mixture genotyping issues [10]. For manual mixture review, these new SWGDAM guidelines introduced a higher "stochastic" threshold for use in a modified CPI (mCPI) mixture interpretation method. After determining locus alleles using the analytical threshold, and establishing that an individual is included, the more stringent mCPI method additionally requires that every allele over the analytical threshold must also reach the stochastic threshold; otherwise the locus cannot be used in the mCPI match statistic. The taller peak height requirement addresses genotype errors by statistically removing ambiguous locus situations where a peak resides in a third state, between the analytical and stochastic thresholds. However, mCPI can discard potentially useful identification data, which lowers match statistics and reclassifies previously interpretable mixtures as "inconclusive".

The Virginia Department of Forensic Science (DFS) implemented the new SWGDAM mixture interpretation guidelines, and reviewed their DNA evidence using stochastic thresholds. In 2011, DFS identified 375 criminal cases in which their stochastic threshold method had produced an inconclusive result or a less informative match statistic [11]. Interested in preserving more identification information, DFS employed a provision in the SWGDAM guidelines (paragraph 3.2.2) that allowed use of a validated "probabilistic genotyping" computer interpretation method [10].

Mathematical modeling can account for quantitative STR data patterns [12]. Combining different amounts of contributor genotypes, along with other variables, produces allele patterns that can be compared with STR data peaks [13]. Incorporating probability into the equations allows a computer to assess the relative likelihood of alternative solutions [14,15]. The result is a genotype probability distribution that is objectively derived from the data, independent of known comparison genotypes. Subsequent comparison of this evidence genotype with a reference genotype, relative to a human population, produces a DNA match statistic that measures identification information. By using all of the quantitative DNA mixture data, and thoroughly considering all feasible genotype alternatives, computer modeling can preserve more identification information than manual review [7].

DFS pursued a probabilistic genotyping approach for their DNA mixture evidence. They arranged for Cybergenetics (Pittsburgh, PA) to apply their validated TrueAllele Casework system to DNA mixture evidence in 144 cases. Cybergenetics produced DNA match reports on 92 evidence items in 72 cases. This is the largest data set on which case reports have been generated for probabilistic genotyping of DNA mixture evidence.

This study describes the results of computer-based probabilistic genotyping mixture interpretation on 101 reported matches, out of 111 genotype comparisons. (A DNA match is defined here operationally as a comparison between an evidence and reference genotype, relative to a population, that gives a reproducible positive match statistic). The 10 comparisons that did not produce a match are also characterized. The study compares the computer's information yield with two methods of manual interpretation on the same evidence items. Previous TrueAllele Casework validation studies have been published on samples of known composition [13,16], as well as on actual casework items [7,17]. This observational study was performed on casework items.

Table 1. Distinguishing features of three different DNA mixture interpretation methods.

		TrueAllele	CPI	mCPI
Peak data	*Approach*	quantitative	qualitative	qualitative
	Scale	continuous	binary	ternary
	Height	used		
	Pattern	used		
	Threshold		analytical	analytical and stochastic
Genotype	*Inference*	probability model	data above analytical threshold	data above analytical threshold
	Representation	allele pairs	alleles	alleles
	Operation	automated	manual	manual
	Inclusion	statistical	alleles	alleles
	Contributor number	assumed		
Statistic	*Comparison*	with genotype	with alleles	with alleles
	Locus	all	inclusion	stochastic inclusion
	Calculation	likelihood ratio	inclusion probability	inclusion probability
	Application	include, exclude or inconclusive	include	include
	Identification	information	inclusion	inclusion

Attributes involving STR data usage, genotype inference and match statistic calculation are shown for the TrueAllele, CPI and mCPI methods.
doi:10.1371/journal.pone.0092837.t001

A companion study has been performed using laboratory synthesized mixtures [18].

The present study compares three interpretation methods for analyzing DNA mixtures from actual casework, specifically, the automated TrueAllele Casework computer system, with the traditional CPI and updated mCPI manual threshold-based methods [19]. The manual methods involve determining and applying a threshold for binary or ternary peak classification, whereas the automated approach uses continuous peak quantities without a threshold (Table 1). The study hypothesizes that the automated system will be more statistically powerful, precisely because it uses more of the data that manual methods discard. If this hypothesis is correct, the automated system should generally reach the same conclusions but infer more powerful match statistics, and resolve cases that the manual methods do not.

The paper begins by describing three methods of DNA mixture interpretation, one automated and two done manually. We then present the case materials used and the interpretation procedures employed. We examine the TrueAllele automation method's reliability, using its inferred match statistics to assess how sensitive, specific, precise and accurate it is. (In Forensic Science, "sensitive" and "specific" describe the reliability of analytical instrumentation. With DNA interpretation methods, they can similarly describe the respective degree of positive or negative identification). We compare how well TrueAllele, CPI and mCPI preserve identification information relative to one another, as measured by their match statistics. We conclude by observing that computer-based DNA mixture interpretation can provide an improvement over current manual forensic processes.

Methods

The DNA samples used in this study were lawfully obtained by DFS in accordance with Virginia code Section 9.1–1101. All personal identifiers were removed from DNA data prior to computer interpretation. The submitted scientific manuscript

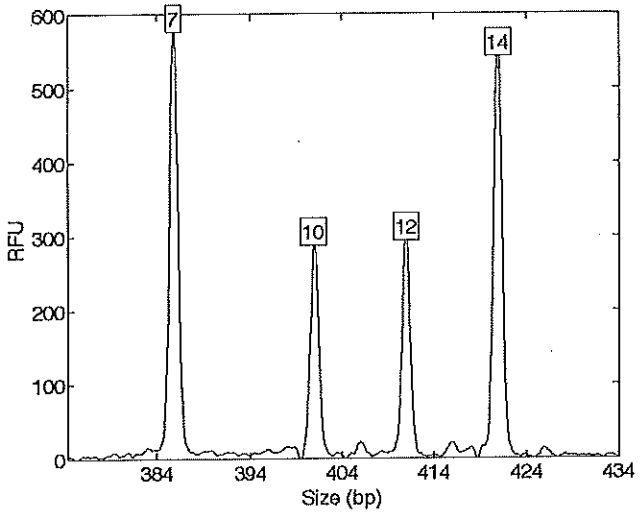

Figure 1. Mixture data. Quantitative DNA mixture data are shown at the Penta E STR locus. The x-axis measures allele fragment size (bp), and the y-axis measures DNA quantity (RFU); a boxed peak number denotes allele length. The two contributor mixture is formed from a 7,14 major genotype and a 10,12 minor genotype. The result is a pattern of peak heights that reflect the underlying genotypes.
doi:10.1371/journal.pone.0092837.g001

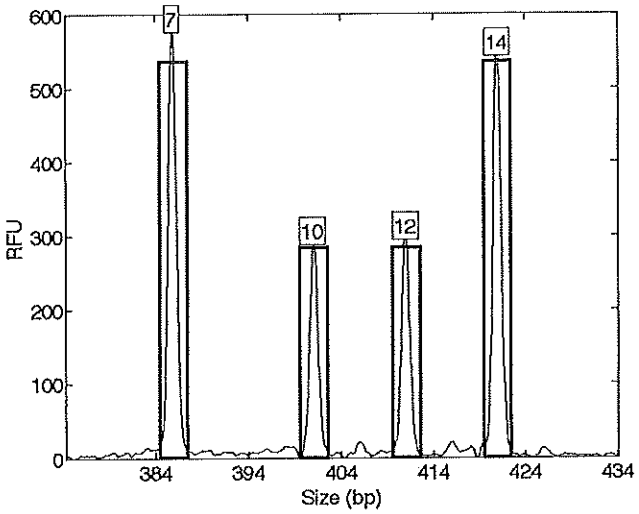

Figure 2. Genotype modeling. Linear combinations of genotype allele pairs can explain the observed quantitative mixture data. Here, a major 7,14 contributor (blue bars) having twice the DNA as a minor 10,12 contributor (green bars) explains the data well, with a high likelihood value. Alternative genotype choices or combinations would not explain the data as well, and thus have lower likelihood.
doi:10.1371/journal.pone.0092837.g002

contains only summary statistics, and discloses no personal or case information.

The DNA mixture interpretation process begins with electronic data signals. These signals are examined to form genotypes. Comparison of an evidence genotype with a reference genotype, relative to a population, can then produce a DNA match statistic.

STR Mixture Data

A STR locus is a length polymorphism, where alleles have different numbers of short DNA units (typically four or five base pairs) that are repeated in tandem [5]. When a polymorphic locus has 15 or more alleles, it provides over a hundred possible genotype values. This genetic variation is useful for distinguishing between people in a population. For example, the Penta E locus on chromosome 15 contains the five base pair repeat unit $(AAAGA)_n$, with $n = 5, 6, \ldots, 24$; these 20 alleles permit 210 distinct allele pairs. (Given n alleles, there are $n(n+1)/2$ possible unordered allele pairings, with n homozygotes and $n(n-1)/2$ heterozygotes. With $n = 20$, there are $20 \cdot 21/2$, or 210 genotype values).

Following DNA extraction and quantification, STR analysis proceeds in two steps. First, PCR amplification with a set of fluorescently labeled primers creates millions of allele copies from the DNA template. Random variation in a 31 cycle PCR process [19] produces natural variation in the quantities of amplified alleles [20]. Second, the allele amplicons are size-separated by capillary electrophoresis, with laser detection of DNA quantity measured in relative fluorescent units (RFU). The amplified allele size and quantity signals are recorded as peaks in an electropherogram (EPG), and saved into a fragment size analysis (.fsa) data file.

Penta E is one of 15 STR loci in the Promega PowerPlex 16 multiplex kit [21]. The example EPG data at this locus show a pattern of allelic peaks, where the x-axis (molecular size) corresponds to the allele's number of repeats and the y-axis (RFU height) relates to allele quantity (Figure 1). The data have

Table 2. Genotype probabilities and LR calculations are shown at the Penta E locus for a minor contributor.

Allele Pair			TrueAllele			CPI			mCPI		
		prior	likelihood	posterior	LR	likelihood	posterior	LR	likelihood	posterior	LR
7	7	4.3%				1	17%		1	67%	
7	10	3.3%	2			1	13%				
7	12	7.1%	2	1%		1	28%				
7	14	1.9%				1	8%		1	30%	
10	10	0.6%	1			1	2%				
10	*12*	*2.7%*	*986*	*98%*	*37*	*1*	*11%*	*4*			*0*
10	14	0.7%				1	3%				
12	12	2.9%	8	1%		1	11%				
12	14	1.6%	1			1	6%				
14	14	0.2%				1	1%		1	3%	
Total				100%			100%			100%	

Three different mixture interpretation methods were used, TrueAllele, CPI and mCPI. Over the sample space of possible allele pairs, each method has a likelihood function and posterior probability distribution. The LR gives the ratio of posterior to prior probability at comparison allele pair 10,12 (italicized row). TrueAllele's greater LR indicates more use of the STR data than CPI. mCPI discarded too much data, and could not yield a match statistic.
doi:10.1371/journal.pone.0092837.t002

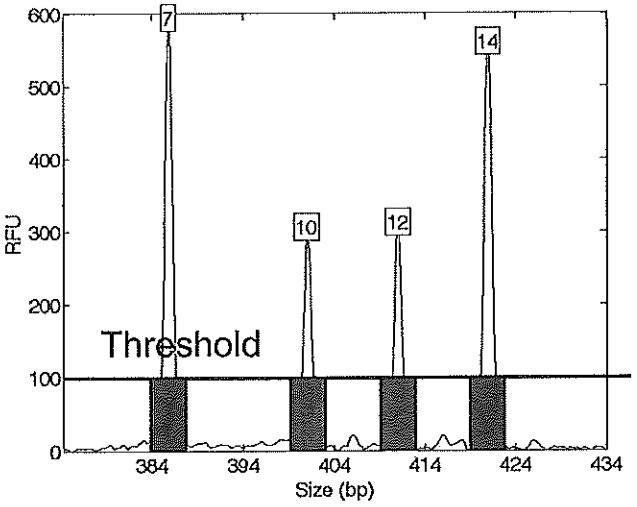

Figure 3. Analytical threshold. The purpose of this threshold is to distinguish allelic signal from background noise. Applying the threshold (red line) reduces the quantitative peaks to all-or-none putative allele events (blue bars). The analytical threshold operation eliminates individual peak heights, as well as their collective pattern.
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two tall peaks for alleles 7 and 14 with heights around 600 RFU, and two shorter peaks at alleles 10 and 12 of height 300 RFU.

Three Interpretation Methods

STR mixture data can be interpreted in different ways, giving rise to different DNA match statistics. Table 1 lists the features of three such methods – the quantitative computer-based TrueAllele approach, as well as the two qualitative human review methods CPI and mCPI. TrueAllele uses all the peak height data on a continuous RFU scale, examining the entire peak pattern to make inferences. Applying an analytical RFU threshold, CPI reduces the peak height quantities to two binary states (allele or not), while mCPI additionally applies a higher stochastic threshold to develop a third state (uncertain).

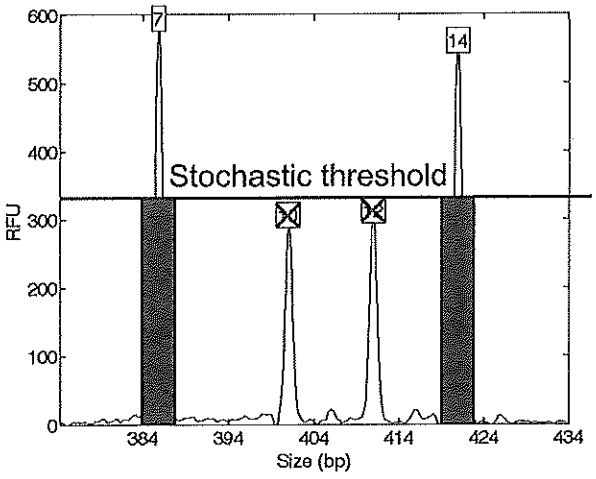

Figure 4. Stochastic threshold. A higher threshold level (red line) is used in manual review to address random peak variation by differentiating more certain (blue bars) from less certain peaks. The stochastic threshold removes more STR loci from statistical consideration, which makes less use of the available data.
doi:10.1371/journal.pone.0092837.g004

Table 3. The range of biological sample types that were found in the 92 evidence items is shown.

Sample type	Count
blood	10
epithelial/skin	30
fingernails	2
hair	1
saliva	4
semen	3
stain	1
touch	41

For each sample type, the table records how frequently that type was seen.
doi:10.1371/journal.pone.0092837.t003

TrueAllele infers genotypes with a probability model that uses a computer to automatically propose peak patterns, and assess how well they explain the quantitative data (Table 1, Genotype). The manual methods infer alleles based on events above a predetermined analytical threshold RFU level, and then assess inclusion. Because TrueAllele separates out the genotypes contributing to a mixture, it can compare evidence genotypes (as probability distributions) with reference genotypes. CPI and mCPI reduce peak height data to "alleles" instead of separating out genotypes, and so compare reference genotypes with evidence data features instead of with inferred genotypes.

TrueAllele's inferred (probabilistic) genotypes can be entered into standard formulae to calculate a likelihood ratio (LR) (Table 1, Statistic). This LR result can give weight to inclusion or exclusion, and so all loci are used in the match statistic [22]. CPI and mCPI first establish an inclusion based on the analytical threshold; loci that do not support an inclusion are not assigned a probability of inclusion. The mCPI statistical calculation will not use a locus that has an uncertain allele whose peak height lies between the analytical and stochastic thresholds.

TrueAllele Genotype Modeling

Many variables are considered in genotype modeling, such as the genotypes of each contributor at every locus, the mixture weights (that sum to 1) of the contributors, the DNA template mass, PCR stutter, relative amplification, DNA degradation and the uncertainties of all these variables. A likelihood function assesses how well particular values of these variables explain the observed quantitative STR data peaks, determining the probability of the (fixed) data conditioned on the (changing) variable values.

Table 4. The first three rows estimate for each number of contributors (first column) how many mixture items (second column) had that contributor number.

	Contributors	Items
Estimate	2	40
	3	65
	4	8
Overlap	2 or 3	16
	3 or 4	3
	2, 3 or 4	1

When an item was consistent with more than one contributor number possibility, that item appears in multiple categories. The last three rows examine overlap situations where the number of contributors (first column) was uncertain, and counts the number of items (second column) in those situations.
doi:10.1371/journal.pone.0092837.t004

Table 5. The frequency distribution of mixture weights as inferred by the computer is shown for the matched genotypes.

Mixture Weight	Count
0.05	3
0.15	13
0.25	5
0.35	12
0.45	18
0.55	12
0.65	11
0.75	12
0.85	12
0.95	4

The binning is done by decile, with each row showing the center of its mixture weight range, along with the number of genotypes in that bin.
doi:10.1371/journal.pone.0092837.t005

With DNA mixture data vector $\mathbf{d}$ (of peak heights and sizes) having K contributors, the primary explanatory variables are the genotypes G, mixture weight W and mass M (of combined allelic fluorescence intensity). An approximate likelihood function containing these variables is

$$\Pr\{\mathbf{d}|G=\mathbf{g},W=w,M=m,...\}=MVN(\mu,\Sigma)$$

where mean pattern vector $\mu=m\sum_{k=1}^{K} w_k\mathbf{g}_k$ and covariance matrix Σ are parameters of a multivariate normal (MVN) distribution, as previously described [7,13]. Pattern μ is constructed as a weighted sum of contributor allele pairs $\mathbf{g}_k$.

We can visually understand this likelihood function as constructing a pattern of allele heights that can be compared with the peak height data. Figure 2 shows a major contributor 7,14 allele pair (blue rectangles of equal height) and a minor 10,12 genotype value (green rectangles of equal height) in a 2:1 mixture ratio. This genotype model is superimposed on the STR peak data, where we see a good fit between the model and data patterns, which corresponds to a high likelihood value. Alternative genotype values and amounts might not explain the data as well (e.g., proposing genotypes 7,10 and 12,15), and would have a lower likelihood.

The posterior genotype probability is proportional to the likelihood value times the prior population probability [23]

$$\Pr\{G=\mathbf{g}|\mathbf{d},W=w,M=m,...\}$$
$$\propto\Pr\{\mathbf{d}|G=\mathbf{g},W=w,M=m,...\}\Pr\{G=\mathbf{g}\}.$$

Bayes theorem [24] requires us to consider all feasible genotype alternatives, even those having little probability. Other variables, such as mixture weight, are similarly framed as posterior

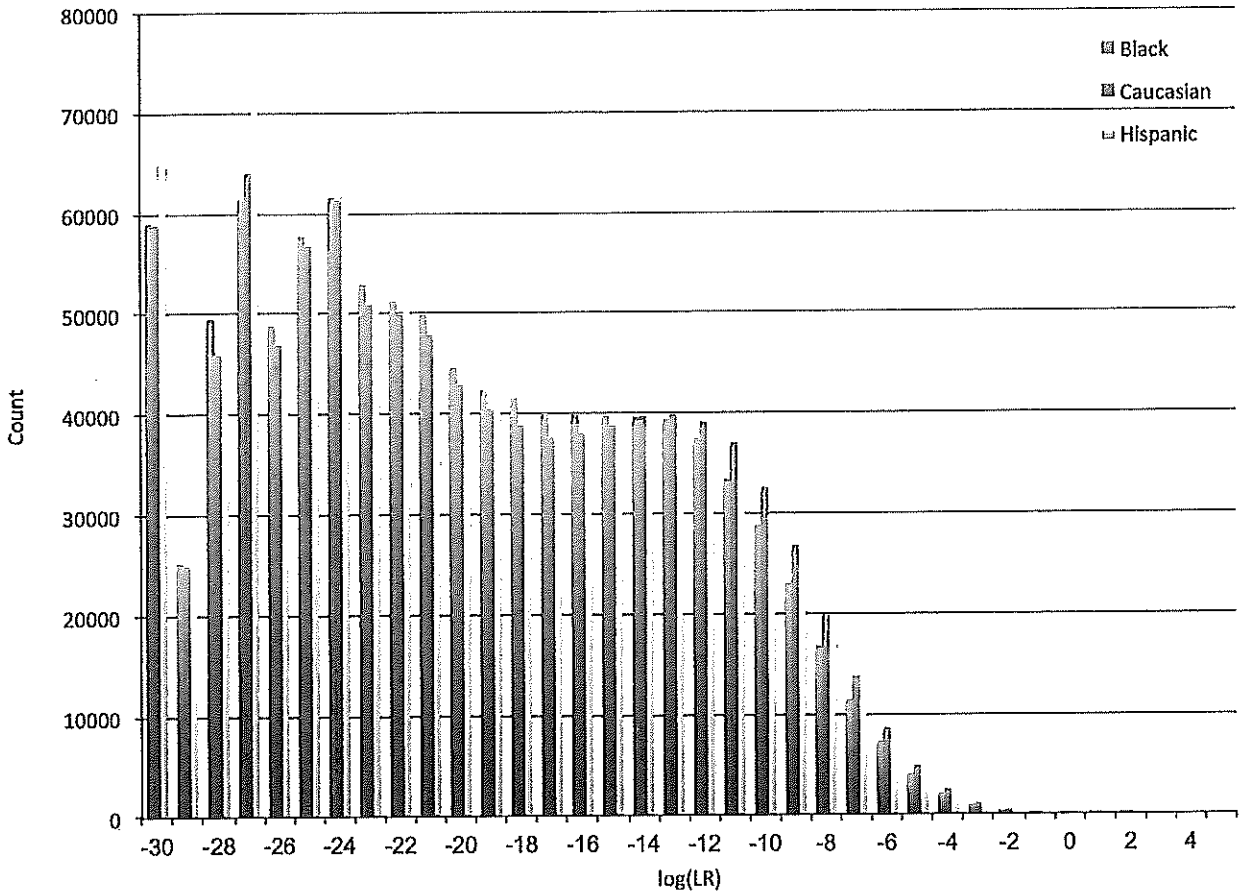

Figure 5. Computer specificity. A histogram shows empirical log(LR) distributions for 101 evidence genotype comparisons relative to 10,000 randomly generated references. There are 1,010,000 data points for each of the three ethnic populations. Note that the negative values are located far to the left of zero.
doi:10.1371/journal.pone.0092837.g005

probability distributions [7]. Since the high dimensional parameter space is vast, the TrueAllele computer conducts a statistical search using Markov chain Monte Carlo (MCMC) to thoroughly sample the joint posterior probability distribution [25,26].

The modeling approach is objective in the sense that only evidence data is used to infer genotypes, without any knowledge of a reference comparison genotype. Proceeding *ab initio* from the data and model eliminates natural examination bias issues that

Table 6. Specificity results (ban) for TrueAllele mixture interpretation log(LR) values, comparing 101 reported evidence genotypes with 10,000 random genotypes from each of three ethnic populations.

n = 3,030,000	Black	Caucasian	Hispanic
Minimum	−30.000	−30.000	−30.000
Mean	−19.467	−19.217	−19.547
Maximum	2.381	2.726	3.782
Standard deviation	6.543	6.723	6.637
Tail distribution	**Black**	**Caucasian**	**Hispanic**
0	39	32	29
1	8	11	9
2	2	1	1
3	0	0	1
log(LR) >0	49	44	40

The average exclusionary LR value was around one over a billion billion. Very few false positives were seen in over three million genotype comparisons.
doi:10.1371/journal.pone.0092837.t006

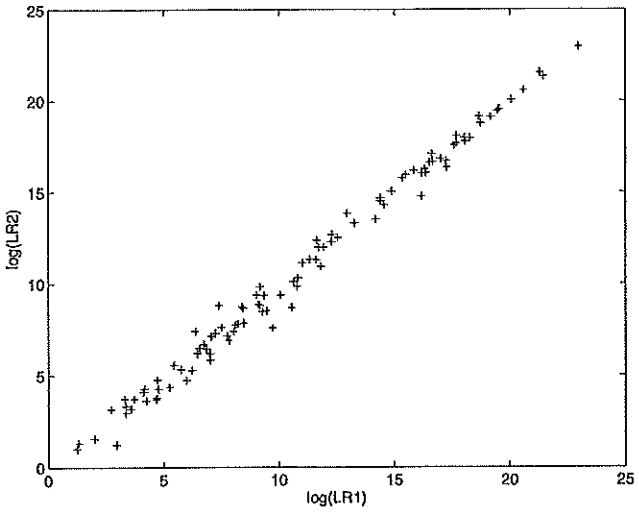

Figure 6. Computer precision. The scatterplot shows log(LR) values for 101 duplicate computer runs on the same evidence. Each point gives the first (x) and second (y) values. The data lie close to the y=x diagonal, which represents exactly replicated results.
doi:10.1371/journal.pone.0092837.g006

may affect other mixture interpretation approaches [27]. The resulting evidence genotype for the minor contributor at this locus concentrated 98% of its probability at allele pair 10,12 (Table 2, TrueAllele).

CPI Allele Inclusion

Inclusion methods of DNA mixture interpretation begin by applying an analytical threshold to the quantitative STR peak data. The Virginia DFS analytical thresholds are specific to each fluorescent dye channel: 73 RFU (blue dye), 84 RFU (green), 75 RFU (yellow) and 52 RFU (red). Peaks above the threshold are designated as "allele" events, while those below are not used (Figure 3).

The inclusion likelihood function assigns 1 to all allele pairs included in the allele list, and 0 otherwise. This CPI likelihood also assumes that all alleles from each contributor are present. With four allele events, for example, there are ten possible allele pairs (Table 2, CPI). Multiplying the prior probability times the 0/1 likelihood values, and renormalizing, gives the CPI genotype probability distribution [28].

The inclusion approach disperses probability over (in this example) ten genotype values. Many of these allele pairs (e.g., 7,7) are not compatible with a minor contributor genotype, based on the peak height data shown. Since the total probability is 1, diverting genotype probability away to infeasible solutions reduces the probability at more likely solutions, and thereby lowers match strength. Starting from highly informative STR data, CPI may reduce considerably the reported identification information, or even eliminate it entirely by dismissing an evidence item as "inconclusive". Inclusion protocols are susceptible to examination bias, since a reference genotype could be considered (e.g., to assess potential allelic dropout) when determining whether to use a locus in a CPI statistical calculation [29].

mCPI Stochastic Threshold

Replicate STR experiments exhibit natural variation in peak height, as described by probability model data variance parameters [13,30]. When interpreting DNA evidence using threshold approaches, stochastic thresholds help to account for this peak pattern variability, which is often more pronounced in low-template samples [8]. A laboratory determines its stochastic threshold through replicate PCR experiments that examine heterozygote allele imbalance and drop out. For example, in following the SWGDAM 2010 guidelines, Virginia DFS set its stochastic thresholds for different capillary injection times as 210 RFU (2s), 320 RFU (5s) and 460 RFU (10s) [19].

The higher mCPI stochastic threshold can make less use of the STR data. In our Penta E mixture example, the 5s injection peak heights of alleles 10 and 12 now fall below the stochastic threshold of 320 RFU (Figure 4). This peak removal can assign essentially zero probability to a 10,12 minor contributor allele pair at this locus in a statistical calculation (Table 2, mCPI). Manual mCPI mixture interpretation would omit Penta E from the cumulative match statistic because of the uncertain alleles 10 and 12, and thus not report the identification information at that locus.

Likelihood Ratio

The likelihood ratio is a standard DNA match statistic [31]. The LR summarizes in one number the impact of STR data on our belief in the identification hypothesis H that an individual contributed their DNA to biological evidence. The base ten

Table 7. The log(LR) DNA match information (ban) for genotype comparisons is shown for three mixture interpretation methods (TrueAllele, CPI and mCPI).

	TrueAllele	CPI	mCPI
Minimum	1.255	0.778	0.301
Median	10.550	6.681	1.857
Mean	11.054	6.825	2.145
Maximum	22.962	16.724	6.447
Standard deviation	5.421	2.217	1.675
N=	111	81	70
Inclusion (≥0)	101	81	53
Persuasive (≥6)	82	54	2
Inconclusive			17

The TrueAllele method preserved more identification information (mean) over a broader range (minimum, maximum) than the two inclusion methods, and produced more inclusions and persuasive match statistics.
doi:10.1371/journal.pone.0092837.t007

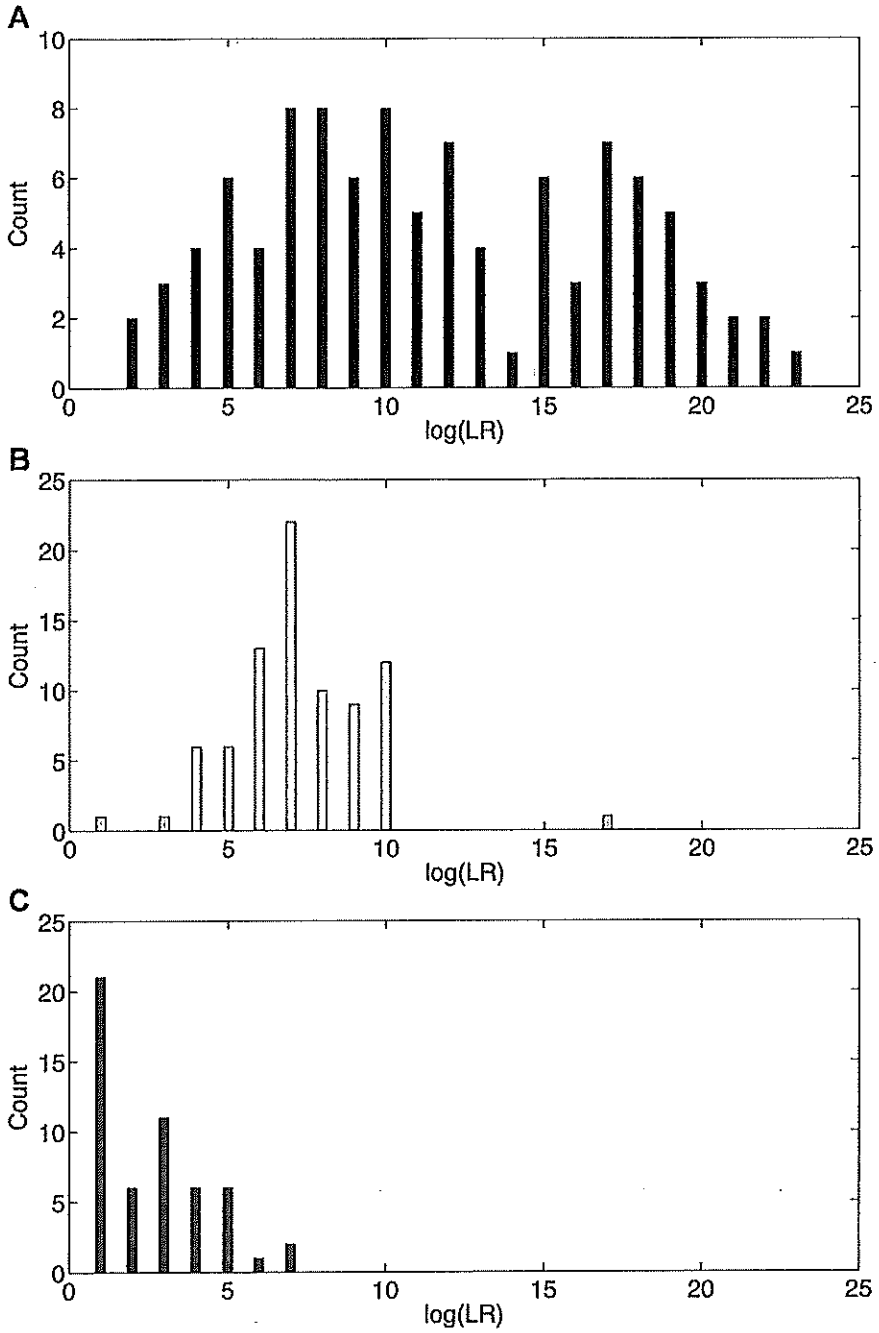

Figure 7. Method sensitivity. Three histograms show the empirical log(LR) distribution for different mixture interpretation methods on the case data. Frequency distribution (**a**) shows TrueAllele inferred genotype match statistics for 101 evidence genotype matches (blue). The (**b**) manual CPI review yielded 81 match statistics (green) that were generally less informative (leftward) and less varied (clustered). The (**c**) 53 mCPI match statistics (red) gave less information and had similar values.
doi:10.1371/journal.pone.0092837.g007

logarithm of the LR expresses identification information in additive "ban" units, and is called the "weight of evidence" [32].

There are several ways to calculate a LR match result, all of which produce the same number [33]. Since our focus here is on genotypes, we note that the LR is the ratio of posterior (after having seen evidence) to prior (the population distribution) genotype probabilities, evaluated at the allele pair of a known reference [34]. For case reporting, we write "a match between the evidence and reference is (some number) times more probable than coincidence". The LR can also account for co-ancestry, the relatedness in populations between all people [35,36].

When a genotype likelihood function accounts for observed quantitative evidence data, a reproducibly inferred LR number can accurately summarize the extent of match between that evidence and a reference. A positive log(LR) provides a weight of evidence supporting a match, a negative log(LR) does not favor a match, while a log(LR) near zero is inconclusive. The LR value is always scientifically meaningful. Scientists sometimes verbally describe a LR using an arbitrary subjective scale [37].

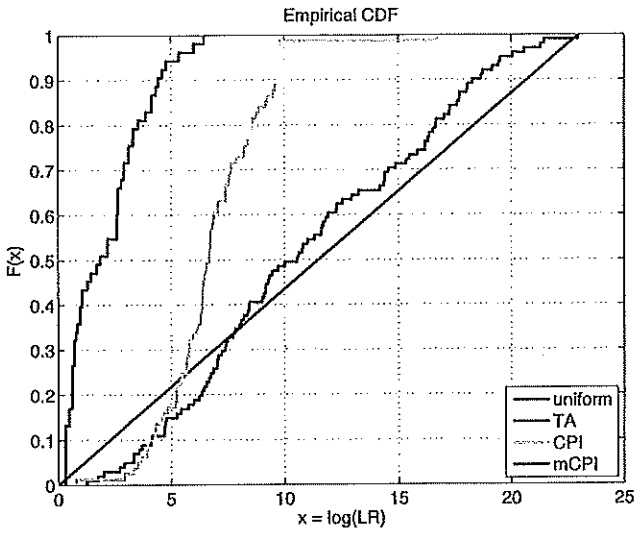

Figure 8. Method comparison. Cumulative empirical log(LR) distributions are shown for uniform probability (black), and for each of the three mixture interpretation methods TrueAllele (blue), CPI (green) and mCPI (red). TrueAllele tracks a uniform distribution over a wide information range, whereas CPI and mCPI do not.
doi:10.1371/journal.pone.0092837.g008

Comparing Three Interpretation Methods

Table 2 shows the prior, likelihood and posterior genotype probabilities of a minor contributor for three different mixture interpretation methods at locus Penta E. The prior probability is the population prevalence of an allele pair (Table 2, prior). The differences between these methods reside in their likelihood functions (Table 2, likelihood):

- The *TrueAllele genotype modeling* likelihood function is a positive real number that describes how well each allele pair hypothesis explains the STR data.
- With *CPI allele inclusion*, the analytical threshold produces a list of included possible allele pairs; these receive a likelihood of 1, and all other values are set to 0.
- Using a *mCPI stochastic threshold* at a higher RFU level forms a possibly shorter list of allele pairs, corresponding to binary 0/1 likelihoods.

A method's posterior genotype probability is the product of its likelihood and the prior, normalized to sum to unity (Table 2, posterior).

The LR is shown (Table 2, LR) as a ratio of posterior to prior genotype probabilities. We see that TrueAllele genotype modeling used peak height information to make a clear distinction between the 7,14 major and 10,12 minor genotype contributors. By ascribing 98% of the probability to genotype 10,12, the continuous computer method produced a LR of 37 (posterior to prior ratio of 98/2.7) that preserved virtually all of the identification information.

The CPI allele inclusion method uses all data peaks above a predetermined analytical threshold to form allele pairs [38]. The LR of the inclusion genotype at known 10,12 relative to the population is 4 (11/2.7), the reciprocal of the inclusion probability. The inclusion method's LR of 4 at this locus is approximately an order of magnitude less than TrueAllele's genotype modeling LR of 37. Multiplying together independent locus inclusion LR values gives the CPI match statistic. The inclusion method is named CPI by its match statistic, also dubbed Random Man Not Excluded (RMNE).

mCPI uses a stochastic threshold to produce a DNA match statistic. mCPI only uses those loci at which all of the peaks are above the stochastic threshold. In our Penta E locus example, the data peaks corresponding to the known 10,12 individual are both under the threshold, setting the mCPI likelihood to zero. Therefore the mCPI posterior probability and LR (from the calculation 0/2.7) of the locus would both be zero, as well. This locus was not used in the mCPI calculation.

Considering all loci in this mixture, TrueAllele's log(LR) was 16.32; the weight of evidence was 7.04 ban for CPI, and 6.00 ban for mCPI. This example illustrates how genotype modeling makes more use of the data to preserve DNA match information, while an already diminished CPI match statistic can be further reduced by the mCPI stochastic threshold. Our study examines this phenomenon on a larger set of Virginia DFS case matches, comparing the three mixture interpretation methods TrueAllele, CPI and mCPI.

Materials

Mixture Data

The Virginia DFS identified DNA mixture cases where computer interpretation could potentially make more use of the STR data than manual review. The selection criteria included having a probative DNA item, possible use of that item as evidence in a criminal trial, an included person of interest, and a need for accurate DNA match information. Items that were easy to interpret manually were not chosen.

The 72 cases spanned a full range of biological evidence, including touch, epithelial cells, blood, saliva and semen (Table 3). These samples are representative of DNA laboratory casework items. The DNA evidence items were all mixtures, most having 3 contributors and some 4 (Table 4), as estimated visually from locus peak counts and patterns.

Table 8. Paired comparisons for positive log(LR) values between TrueAllele (TA) and CPI.

N = 81	TA	CPI	TA – CPI	test	p-value
Mean	11.623	6.825	4.798	t = 8.396	1.350×10^{-12}
Median	10.816	6.681	4.135	W = 3047	6.664×10^{-11}
$r = 0.2999$					
$r^2 = 0.0900$					

Significance tests were done for means (Student t) and medians (Wilcoxon signed rank W). Correlation coefficients (r) and coefficient of determinations (r^2) are shown. TrueAllele was significantly more informative than CPI.
doi:10.1371/journal.pone.0092837.t008

Table 9. Paired comparisons for positive log(LR) values between TrueAllele (TA) and mCPI.

N = 53	TA	mCPI	TA – mCPI	test	p-value
Mean	12.883	2.145	10.738	t = 15.147	1.040×10^{-20}
Median	12.537	1.857	10.679	W = 1431	2.386×10^{-10}
$r = 0.2945$					
$r^2 = 0.0867$					

Significance tests were done for means (Student t) and medians (Wilcoxon signed rank W). Correlation coefficients (r) and coefficient of determinations (r^2) are shown. TrueAllele was significantly more informative than mCPI.
doi:10.1371/journal.pone.0092837.t009

The mixture weights as calculated by TrueAllele were evenly distributed between 10% and 90% (Table 5). Statistically comparing this empirical mixture weight distribution with a uniform probability distribution gave a Kolmogorov-Smirnov test [39] statistic of 0.1079, whose p-value (0.2220>0.05) showed no significant difference between the distributions.

Virginia DFS generated STR data using the Promega PowerPlex 16 kit (Madison, WI), analyzed on an Applied Biosystems 3130×l Genetic Analyzer (Foster City, CA). The DFS case materials included electronic.fsa data files from the sequencer, their own case reports and case context descriptions. DFS sent these electronic materials via secure file transfer protocol (sFTP) to Cybergenetics during the latter half of 2011. The data files were organized in batches for computer processing.

TrueAllele System

Cybergenetics TrueAllele Casework is a computer system for resolving DNA mixtures into their component genotypes [40]. Written in the MATLAB programming language, the computer uses MCMC sampling [25] to solve a hierarchical probability model [23]. (In this paper, a "computer" always refers to TrueAllele Casework software running on either a client or server computer). A human operator uses VUIer (Visual User Interface) client software that interfaces over a network with a server that hosts a database and parallel processors running interpretation software.

TrueAllele divides DNA identification into two phases [41]. The computer first infers genotypes from the evidence data. The inference is objective in that it has no knowledge of downstream comparison reference genotypes. Afterwards, a comparison can be made between an inferred evidence genotype and a reference, to calculate a LR [31] relative to a population. Separating mixture data into single source-like genotypes can make results easier to explain [33] and simpler to report [42].

Procedure

TrueAllele Processing

For each received batch of cases, Cybergenetics processed the.fsa files in the TrueAllele Analyze module to assess data quality. For computing efficiency, EPG peaks below the baseline noise level of 10 RFU were not used (since they do not affect the results). The quality-checked quantitative peak data were then uploaded to a TrueAllele database.

A trained first TrueAllele operator processed a case by downloading from the database the electronic data for all evidence items. The operator examined the EPG signals, and estimated the number of contributors for each evidence item based on the number of peaks observed at each locus. If relevant and available, an assumed reference could be used. (For example, with an intimate sample from a sexual assault, assuming the victim's genotype as a known contributor to the mixture is forensically meaningful). Appropriate DNA interpretation case questions were uploaded as "requests" from the VUIer to the TrueAllele database for processing.

Following this initial processing, an experienced second TrueAllele operator then reviewed the computer results, and determined whether further analysis would be required. Such additional TrueAllele analyses could entail assuming a different number of contributors, considering DNA degradation, or repeating the question using more computer processing time. When the number of contributors was ambiguous, multiple contributor assumptions were tested; the assumed number of contributors (when there are enough) does not have a major effect on the inferred genotypes or match statistics. Reportable DNA results were replicated in two or more independent computer runs.

Case Reporting

A reporting scientist examined all the computer results in a case. After careful review of the replicated genotypes, together with the data and mixture weights, a concordant genotype subset was identified. Concordant genotypes had similar probability distribu-

Table 10. Paired comparisons for positive log(LR) values between CPI and mCPI.

N = 52	CPI	mCPI	CPI – mCPI	test	p-value
Mean	7.069	2.180	4.889	t = 17.417	4.082×10^{-23}
Median	6.720	2.024	4.696	W = 1378	3.497×10^{-10}
$r = 0.5188$					
$r^2 = 0.2692$					

Significance tests were done for means (Student t) and medians (Wilcoxon signed rank W). Correlation coefficients (r) and coefficient of determinations (r^2) are shown. CPI was significantly more informative than mCPI.
doi:10.1371/journal.pone.0092837.t010

Table 11. Results are shown for ten genotype comparisons where TrueAllele did not report a match, and five others having a small LR value under a thousand.

Interpretation Method			Data Observations					
TrueAllele	**CPI**	**mCPI**	**allele dropout**	**allele overlap**	**low peaks**	**peak imbalance**	**infeasible mixture**	**infeasible pattern**
−10.64			3	4	1			1
−6.52			4	3	1			1
−5.05			4	3	1	1		1
−4.87				3		1	1	1
−4.86	3.48		4		1		1	
−3.22	6.04	6.34		2			1	1
−2.99	4.23		2		1		1	1
−2.18			2		1		1	
−1.41	4.08		1		1		1	
−0.67	2.95	0.60	1	2	1			
1.26	3.96		1	4			1	
1.76			1			1	1	
2.01			2	8		1	1	
2.71			2				1	
2.94				8		1		

Allele dropout and *allele overlap* record the number of locus occurrences.
Allele dropout occurs when a reference allele does not appear at all in the evidence data.
Allele overlap occurs when known contributors and the reference share alleles.
Low peaks: All had reference-related allele peaks <100 RFU. A 1 indicates peaks <50 RFU.
Peak imbalance: a 1 indicates heterozygote imbalance under 60% at reference alleles.
An *infeasible mixture* (1) has an inconsistent mixture weight across loci.
An *infeasible pattern* (1) cannot be constructed quantitatively from contributor genotypes.
Each comparison row gives log(LR) match statistics (ban) for three mixture interpretation methods, and lists observations about how the evidence data interacted with the reference genotype.
doi:10.1371/journal.pone.0092837.t011

tions, mixture weights and Kullback-Leibler (KL) statistics [43]. These properties all measure the inferred genotype, and are independent of any reference comparison or match result. The scientist chose a representative genotype from this concordant set.

In the VUIer program, the TrueAllele scientist indicated the three genotypes (evidence, reference and population) needed to calculate a LR. Virginia's databases of Black, Caucasian and Hispanic populations were used, and the co-ancestry coefficient was set at 1%. All three LRs were reported; for comparison purposes in this study, we conservatively took the smallest of the three match statistics.

Match Statistic Collation

Cybergenetics processed the data, and prepared DNA match reports for 72 cases requested by DFS. These cases encompassed 92 items of evidence and 111 comparisons to reference individuals. TrueAllele LR values were collated from these reports. DFS had independently conducted manual mixture calculations on most of the reported TrueAllele matches. These CPI and mCPI match statistics were collected and recorded in a LR format.

A DFS forensic examiner assessed DNA evidence to determine whether a person of interest could be eliminated from the data. This assessment considered the number of contributors, sample type, DNA quantity, potential drop out, and other factors. When the data were inconclusive or the person had been eliminated, no match statistic was calculated.

Results

We assessed the reliability of DNA mixture interpretation methods through information metrics based on log(LR). The data set comprised 111 computer-inferred evidence genotypes and match statistics; our focus is on the 101 reported matches. We consider in turn how specifically, precisely and sensitively the TrueAllele system performs. Information comparisons with CPI and mCPI methods are possible because formally these manual methods are LRs [28].

Recall that the identification hypothesis H is that a particular individual contributed their DNA to biological evidence. The alternative hypothesis ~H is that they did not, i.e., that the DNA was left by someone else. Forensic science standardly approximates ~H with a random man hypothesis that the DNA contributor is an unrelated person selected at random from a genotype population [31].

TrueAllele Specificity

Specificity measures the extent to which a mixture interpretation method does not misidentify the wrong person. Since identification information is expressed through the log(LR), let X be a real-valued random variable of log(LR) values. We want to understand the TrueAllele distribution of $Pr\{X=x \mid \sim H\}$, the information X conditioned on randomly selected genotypes (that are not contributors to the mixture). The specificity statistic $Pr\{X>0 \mid \sim H\}$ then tells us how frequently a positive log(LR) occurs by chance.

Toward this end, we generated ten thousand random genotypes from each of the three Virginia ethnic populations. This generation was done by randomly selecting alleles in proportion to their prevalence in the population database. We compared the 101 matching TrueAllele-inferred evidence genotypes to these random reference genotypes, relative to the appropriate population, to calculate log(LR) values; the co-ancestry coefficient was set to 1%. These values provided a representative log(LR) sampling of over a million nonmatching comparisons for each population.

The resulting empirical $Pr\{X=x \mid \sim H\}$ distribution is shown in Figure 5, where the k^{th} bin aggregates the log(LR) values for the interval $k \leq x<k+1$. A negative log(LR) value means that a coincidental match is more probable than the evidence matching the reference genotype. TrueAllele's log(LR) distribution is highly negative, with an average value around −19.5 (Table 6). Thus, for noncontributors, the computer-inferred probability of an evidence genotype is generally much less than the population frequency.

The specificity value $Pr\{X>0 \mid \sim H\}$ was estimated by counting the fraction of positive log(LR) outcomes. For all three ethnic populations, TrueAllele's false positive rate was less than one in twenty thousand (Table 6, tail distribution). The rate for $X>3$ was under one in a million, and no false positives were seen beyond that level. The results were essentially the same when reference genotypes were randomly generated using co-ancestry coefficients ranging from 1% to 5% (data not shown).

TrueAllele Precision

TrueAllele's genotype model has hundreds of variables. Therefore the (largely continuous) probability model cannot be solved directly by brute force integration or enumeration. Instead, MCMC computing is used to statistically sample from the joint posterior probability distribution, a standard numerical solution for high-dimensional hierarchical models. Such methods exhibit sampling variation between independent computer runs.

Precision describes a method's reproducibility on the same data. To measure precision, we examined the identification information obtained in duplicate computer runs of the 101 matching genotypes. The observed log(LR) pairs are shown in Figure 6, where the scatterplot shows the points clustering near the $y=x$ diagonal line. Precision can be quantified by calculating within-group standard deviation [7], which is the mean square variation over replicate computations. For the set of genotype matches, we found a precision of 0.305 ban. So, on average, repeated TrueAllele LR values vary by a factor of 2 ($10^{0.305}$) standard deviations.

The log(LR) variation between computer runs was generally greater at medium LR values having logarithms between 5 and 10 (Figure 6). When the LR was small, so were the inter-run deviations. With large LRs, the highly informative genotypes were very reproducible. Statistical tests for heteroscedasticity (Breush-Pagan, White) were not significant ($p>0.05$).

TrueAllele Sensitivity

Sensitivity measures the extent to which a mixture interpretation method identifies the correct person. We therefore examine $Pr\{X=x \mid H\}$, the log(LR) distribution conditioned on the identification hypothesis H. In this observational case study, we want reassurance that H is true, so that the reference genotype actually contributed to the mixture evidence.

The preceding specificity results demonstrated that the false positive rate $Pr\{X>0 \mid \sim H\}$ of TrueAllele's mixture interpretation under the noncontributor hypothesis was less than 0.005%. (This is ten times smaller than the highly reliable 0.05% error rate for dual manual review of easily interpreted single-source reference samples [44]). Moreover, beyond small log(LR) levels around 3 ban, no false positives were seen in millions of comparisons. Indeed, in experimental studies based on samples of "known" composition [13,16,45,46], TrueAllele is used to rectify laboratory errors in genotype composition and mixture weight. Since the method's high specificity assures identification hypothesis H with considerable certainty, we can safely examine the $Pr\{X=x \mid H\}$ sensitivity distribution of positive log(LR) values.

The TrueAllele log(LR) distribution of the 101 reported matches is shown in Figure 7a. For each genotype comparison, we took the smallest of the three ethnic population LR values and used a co-ancestry correction of 1%. The log(LR) values ranged from 1.255 to 22.962, with a mean value of 11.054 ban (Table 7, TrueAllele). As expected, the matching DNA evidence evenly spanned the entire range of positive identification information, from zero to full single-source levels beyond 20 ban. This breadth of scores was also seen in the large standard deviation of 5.421 ban. Of the 101 reported matches, 82 had a DNA statistic exceeding a million, which is a level that people may find persuasive [47].

More accurate genotype modeling employs a likelihood function that better explains the data, and so tends to produce a higher LR (relative to less accurate modeling) when there is a true match. However, the actual LR value depends on the genotype model, thus some other measure of accuracy is needed. Over a large ensemble of DNA mixtures having randomly distributed mixture weights (Table 5) and DNA amounts, one would expect to observe uniformly distributed identification information. So one measure of accuracy is the degree to which a method's empirical log(LR) distribution resembles a uniform distribution.

A uniform probability density function (PDF) is a constant horizontal line. TrueAllele's empirical PDF appears relatively constant across its range of observed log(LR) values (Figure 7a). A better comparison is made using a cumulative distribution function (CDF); for a constant PDF value, the CDF is a straight line moving from 0 up to 1 (Figure 8, black). The computer's empirical CDF forms a reasonably straight line (Figure 8, blue), similar to the uniform CDF (Figure 8, black). The Kolmogorov-Smirnov (K–S) test can statistically assess whether two probability distributions are the same. With a KS value of 0.1059, the p-value (0.2149>0.05) showed no significant difference between TrueAllele's empirical log(LR) distribution and a uniform distribution, providing statistical support for the system's accuracy.

Threshold Methods

The two threshold-based manual methods produced less informative DNA statistics that were distributed differently than the computer's 101 genotype modeling positive log(LR) results. On 81 comparisons, the CPI manual method yielded matches with a mean log(CPI) value of 6.825 ban (Table 7, CPI). The mCPI stochastic threshold method gave 53 matches with a 2.145 ban average, and 17 inconclusive results where a match statistic could not be calculated (Table 7, mCPI). Frequency plots of the log(CPI) and log(mCPI) distributions show a pronounced leftward shift for these two match statistics (Figures 7b and 7c, relative to 7a). The match information range narrowed, with standard deviations of 2.217 and 1.675 ban, respectively.

We can again use the Kolmogorov-Smirnov statistic to test the accuracy of these two manual methods. The empirical CDFs of inferred log(LR) values for both CPI (Figure 8, green) and mCPI (Figure 8, red) are seen to deviate from a uniform distribution (Figure 8, black). For CPI, KS = 0.5609 ($p = 1.8856 \times 10^{-22}$), demonstrating a significant difference between CPI's log(LR) CDF and the uniform distribution. Similarly with mCPI, KS = 0.7352 ($p = 1.1316 \times 10^{-25}$), showing a significant difference between mCPI's log(LR) CDF and the uniform distribution. The nonuniform clustering of CPI and mCPI log(LR) values (Figures 7b and 7c; Figure 8, green and red), statistically confirmed by the KS tests, does not support the accuracy of threshold methods.

Comparison of Methods

The numerical differences in average log(LR) between the three interpretation methods were statistically significant (Tables 8–10). TrueAllele preserved the most information, CPI kept less, and mCPI retained the least. These results are not surprising [7]: threshold methods make less use of the data [48], higher thresholds further reduce information, and the study's case criteria selected for items having low mCPI values. The correlations are also of interest.

The TrueAllele genotype modeling method showed a significant improvement over the older CPI allele inclusion method (Table 8). The mean log(LR) difference was 4.798 (Student $t = 8.396$, $p = 1.350 \times 10^{-12}$), and the median difference was 4.135 (Wilcoxon sign rank $W = 3047$, $p = 6.664 \times 10^{-11}$); both differences exceed four orders of magnitude. There is only a weak correlation ($r = 0.2999$) between the methods, and the small coefficient of determination ($r^2 = 0.0900$) leaves over 90% of the variance unexplained. To the extent that TrueAllele quantitative modeling measures identification information, the CPI binary allele inclusion method is measuring something else.

TrueAllele also showed a significant improvement over the newer mCPI allele inclusion approach (Table 9). Here the mean log(LR) difference was 10.738 ban ($t = 15.147$, $p = 1.040 \times 10^{-20}$), and the median difference was 10.679 ban ($W = 1431$, $p = 2.386 \times 10^{-10}$). The 10 ban difference is a factor of ten billion in DNA match statistic. The weak correlation ($r = 0.2945$) and small coefficient of determination ($r^2 = 0.0867$) again leaves over 90% of the variance unexplained. Since TrueAllele quantitatively measures identification information, the mCPI stochastic threshold method apparently measures some other data attribute.

Switching from allele inclusion to stochastic thresholds significantly reduced the match statistic (Table 10). The mean log(LR) difference between CPI and mCPI was 4.889 ban ($t = 17.417$, $p = 4.082 \times 10^{-23}$), and the median difference was 4.696 ban ($W = 1378$, $p = 3.497 \times 10^{-10}$), which is a match statistic ratio of over ten thousand. There is some correlation ($r = 0.5188$) between CPI and mCPI, but the small coefficient of determination ($r^2 = 0.2692$) does not explain over 70% of the variance. Stochastic thresholds seem to measure inclusion in a different way than does CPI.

These concordant multi-method match results increase confidence in the sensitivity experimental design, where reference genotypes were considered to be present in their respective DNA mixture items. Each of the three interpretation methods works differently, is accepted by courts as reliable criminal evidence, and was calculated independently in the study. In each pairwise comparison, the methods independently agreed on all matches ($N > 50$) and gave positive identification information. These pairwise consensus results were obtained on highly reliable data subsets of the more readily interpretable mixtures.

TrueAllele Conservatism

Out of 111 TrueAllele genotype comparisons, 10 gave a negative log(LR) value, and so did not produce a positive match result (Table 11, first 10 rows). This often occurred when a reference sample allele was not seen as an STR peak in the evidence data, which could be explained by either exclusion or allele dropout. Dropout at a locus usually yields a negative log(LR) value for that locus, which the computer must tally in its joint match statistic. Other STR features that can confound a match between the evidence data and a reference sample are allele overlap, low peaks, peak imbalance, infeasible mixture combinations and an infeasible mixture pattern.

There were 5 genotype comparisons where CPI indicated a match, but the computer found no statistical support (Table 11, TrueAllele <0, CPI >0). Laboratory reexamination of these items agreed with the computer's conclusions. Since the threshold methods did not use peak height information, they supported inclusions whose genotype mixture combinations were incompatible with the quantitative data. Manual mixture interpretation statistics may omit loci that do not demonstrate inclusion, and reported loci can only add positive log(LR) values. The TrueAllele computer, on the other hand, must use all the loci, with negative results at a locus decreasing the total weight of evidence.

Five genotype comparisons gave a small positive TrueAllele log(LR) value of under 3 ban (Table 11, last 5 rows). CPI produced a match statistic in one of these cases, while mCPI provided no statistics. Three of the five items showed considerable allele overlap, which the computer could mathematically resolve better than inclusion methods. There were fewer low peaks and greater peak imbalance in these data, relative to the negative match results. The last column shows that TrueAllele can distinguish between quantitatively feasible and infeasible mixture patterns, while CPI and mCPI may not.

Discussion

Modern criminal justice requires rapid and reliable processing of DNA evidence. Reliability is the basis of admissible evidence, and entails sensitivity, specificity and precision. However, when confronted with complex mixtures or touch DNA, manual review can become a challenging task. Since such mixtures may constitute the bulk of biological evidence found in serious crimes such as sexual assault or homicide, effective interpretation of these data is needed.

In this casework study, the newly adopted mCPI stochastic threshold method produced results in 53 of 70 DNA match comparisons, finding an average match statistic of 140 (Table 7, mCPI, as $10^{2.145}$). The previously used CPI threshold method had greater sensitivity in 81 inclusions on this data set, for an average match statistic of 6.68 million. TrueAllele computer interpretation provided 101 match statistics, with an average LR of 113 billion. The genotype modeling reported on more of the evidence than did threshold methods, and preserved more DNA identification information.

TrueAllele mixture interpretation does not always increase a DNA match statistic. In this study, the computer's statistics were lower than the corresponding human CPI values in 15 reported matches [49]. Moreover, the computer found no statistical support for a match in 10 cases, including 5 where CPI gave an inclusionary match statistic. While the system does find more matches and computes stronger statistics on average, it examines DNA evidence objectively without introducing bias that may favor the prosecution or defense.

In addition to increased average sensitivity, TrueAllele also maintains excellent specificity. The computer's LR can quantify negative match information, unlike manual interpretation methods that are restricted to positive (logarithmic) values. We examined several million genotype comparisons between computer-inferred evidence and randomly generated references, and found a false positive rate of under 0.005%. The negative match information in this simulation experiment had a log(LR) averaging around −19.5 ban.

TrueAllele calculates DNA match statistics with precision. Replicate computer runs on the same evidence data showed a within-group log(LR) standard deviation of 0.305 ban. Thus, independent runs on the same evidence item gave statistically similar DNA match statistics that were usually within an order of magnitude.

To assess accuracy, (logarithmic) match statistic distributions were examined. Across an entire ensemble of matches, the random sampling inherent in casework should produce a uniform log(LR) distribution. TrueAllele's match distribution was statistically uniform, which lends support to the overall accuracy of its LR values. However, the manual methods each clustered around their average match value, and so did not exhibit a uniform distribution that would support their accuracy.

DNA, whether single source or complex mixture, can provide evidence that implicates criminals and exonerates the innocent. Current manual review of DNA mixture data applies thresholds that can discard valuable data and understate the evidential import of the identification information. As demonstrated in this casework comparison study, TrueAllele computer interpretation more effectively preserves DNA evidence and match information, relative to CPI and mCPI methods that use thresholds. Both prosecutors and defense attorneys may benefit from use of this validated computer technology to review complex DNA mixture evidence.

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Author Contributions

Conceived and designed the experiments: MWP LSW. Performed the experiments: KD JH. Analyzed the data: MWP SG. Contributed reagents/materials/analysis tools: MWP LSW. Wrote the paper: MWP KD SG.

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EXHIBIT 8

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Simplification of complex DNA profiles using front end cell separation and probabilistic modeling

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ABSTRACT

Forensic samples comprised of cell populations from multiple contributors often yield DNA profiles that can be extremely challenging to interpret. This frequently results in decreased statistical strength of an individual's association to the mixture and the loss of probative data. The purpose of this study was to test a front-end cell separation workflow on complex mixtures containing as many as five contributors. Our approach involved selectively labelling certain cell populations in dried whole blood mixture samples with fluorescently labeled antibody probe targeting the HLA-A*02 allele, separating the mixture using Fluorescence Activated Cell Sorting (FACS) into two fractions that are enriched in A*02 positive and A*02 negative cells, and then generating DNA profiles for each fraction. We then tested whether antibody labelling and cell sorting effectively reduced the complexity of the original cell mixture by analyzing STR profiles quantitatively using the probabilistic modeling software, TrueAllele® Casework. Results showed that antibody labelling and FACS separation of target populations yielded simplified STR profiles that could be more easily interpreted using conventional procedures. Additionally, TrueAllele® analysis of STR profiles from sorted cell fractions increased statistical strength for the association of most of the original contributors interpreted from the original mixtures.

1. Introduction

One of the biggest challenges with DNA evidence is the presence of cell populations from multiple contributors which can result in decreased statistical strength of STR profile interpretation and, potentially, loss of evidence. Many methods have been developed to separate contributor cell populations prior to DNA profiling including microfluidic manipulations [1], laser capture microdissection [2], and flow cytometry based techniques such as fluorescence activated cell sorting (FACS) [3,4]. However, one limitation of these approaches is that they have largely been demonstrated on mixtures containing only two contributors and/or have been applied to fresh or uncompromised mixture samples. Although probabilistic genotyping systems can perform analyses on mixtures that contain three or more contributors which are superior to human analysis [5,6], limits remain as to the number of contributors that can be successfully disentangled [7]. This is particularly in true for mock casework samples that display stochastic imbalances that impact low level contributors, and create allelic and locus drop-out [8]. Therefore, there is still considerable need for front-end techniques that can reduce the complexity of mixtures with three or more individuals prior to DNA analysis and facilitate the generation of single or near single source STR profiles.

The purpose of this study was to test a workflow for resolving complex biological mixtures that combines front-end cell separation with probabilistic genotyping of the simplified sorted cell fractions. A similar approach has been previously demonstrated with laser capture microdissection as the front end separation approach for enhanced interpretation of buccal cell mixtures containing two contributors in equal ratios [9]. We have built upon this work by processing two-, three-, four- and five-contributor mixtures where only one cell type, blood, is present. Front-end separation was accomplished using antibody probe labelling and Fluorescence Activated Cell Sorting (FACS), a high-throughput, non-destructive cell separation technique previously described for forensic applications [3,4,10,11]. The abundance of antigen targets on white blood cells and average DNA yield make this a useful sample system for investigating this workflow. Additionally, complex blood mixtures may be encountered in forensic casework following homicides with multiple victims, mass disasters, or terrorism incidents.

We employed fluorescently labeled antibody probes targeting the

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A*02 allele of the Human Leukocyte Antigen (HLA) Complex to selectively label individual contributor cell populations in a mixture that were recovered from dried whole blood stains. Cell populations were then physically sorted into two fractions, A*02 positive and A*02 negative (referred to as 'P2' and 'P3', respectively), each of which contained a simplified subset of contributors from the original mixture. The unsorted and sorted fractions were subjected to STR profile analysis and both human and software interpretations using the TrueAllele® Casework System ('TA') for probabilistic modeling. Probabilistic interpretations were compared to traditional analyst assessments using standard caseworking protocols.

2. Materials and methods

2.1. Blood sample preparation

Human whole blood samples (n = 9) were obtained from the Tissue and Data and Acquisition and Analysis Core Facility at Virginia Commonwealth University pursuant to Institutional Review Board protocol #870. Blood samples were screened for the HLA-A*02 allele as previously described [3]; four were HLA-A*02 positive (sample IDs 93, 96, 103, 106) and five were HLA-A*02 negative (sample IDs 94, 95, 104, 105, 107). Multiple contributor blood mixture samples of two to five donors were prepared in the ratios (volume:volume) shown in Table 1. Next, 500 µl of each whole blood mixture was dried in a petri dish and incubated at room temperature for approximately 16 h. After the incubation, cells were eluted from the surface by pipetting 1 ml of 1x Phosphate Buffered Saline solution into the petri dish and transferring the cell solution into a 1.5 ml microcentrifuge tube. Samples were then subjected to red blood cell lysis using the Ammonium-Chloride-Potassium (ACK) lysis buffer (Thermofisher Scientific, Waltham, MA). A 50 µl aliquot of each lysed mixture was retained for the unsorted samples and the remainder of each mixture was labeled with FITC-conjugated anti-human HLA-A*02 antibody (BioLegend, San Diego, CA). As part of our initial optimization experiments, we tested three different concentrations of antibody probe: 5 µg, 2 µg, and 0.5 µg (per 30,000 cells). No appreciable differences in the proportion of hybridized cells were observed between 5 µg and 2 µg samples (Figure S1). Five micrograms was used for all hybridization experiments. Mixtures were then processed using FACS to produce the sorted samples as described in [3]. Untreated blood for each of the nine contributors was used for donor reference samples.

2.2. Fluorescence activated cell sorting (FACS)

Fluorescence activated cell sorting (FACS) was performed using a BD FACS Aria II (Becton Dickenson, Franklin Lakes, NJ) in the Flow Cytometry Core Laboratory on the Medical College of Virginia campus of VCU. FACS separation of antibody-labeled white blood cells was accomplished using a 488 nm laser and gating criteria for discrimination of HLA-A*02-labeled and HLA-A*02-unlabeled cells into the P2 and P3 fractions, respectively.

Table 1
Contributors and ratios for each mixture.

Number of Contributors	Mixture Ratios (vol:vol)	Contributors in Mixture [1]
2	1:1	93(+):94(-)
2	1:1	95(−):96(+)
3	1:1:1	105(−):106(+):107(−)
3	1:1:2	105(−):106(+):107(−)
4	1:2:2:3	103(+):104(−):106(+):107(−)
5	1:1:1:1:1	103(+):104(−):105(−):106(+):107(−)

[1] Contributors are listed in the same order as the mixture ratios. "+" or "-" indicates whether donor cell populations exhibited interactions with the HLA-A*02 antibody.

2.3. DNA extraction

DNA extraction was performed using the DNA IQ™ system which was previously validated for low level samples [12]. All DNA purification reagents were provided in the DNA IQ™ kit (Promega, Madison, WI). Briefly, samples were placed in 1.5 ml microcentrifuge tubes and cell lysis was performed in 160 µl of a Proteinase K buffer (TNE, 2.5% Sarkosyl), 20 µl of 0.39 M Dithiothreitol (DTT), and 20 µl of 20 mg/ml Proteinase K). Samples were incubated at 56 °C for 2 h, then substrate material was removed to a spin basket in the sample tube and centrifuged at 10,000 x g for 5 min to remove excess liquid. DNA preparations of the blood mixture and reference samples were also performed using the Biomek®NX^{P} Automation Workstation (Beckman Coulter, Inc., Indianapolis, IN) following the same process but automated. The purified DNA was stored at 4 °C.

2.4. DNA quantification

DNA was quantified by real-time PCR (qPCR) using the Plexor® HY System (Promega) in a MX3005P™ Quantitative PCR instrument (Stratagene, Santa Clara, CA) equipped with Plexor® HY Analysis software, as detailed in [13]. The Plexor® HY System (Promega, Madison WI) simultaneously quantifies human and male DNA and amplifies an internal positive control that may indicate sample inhibition.

2.5. STR amplification and analysis

STR amplification of extracted DNA was performed using the PowerPlex® Fusion System (Promega, Madison, WI) in a GeneAmp 9700 thermal cycler (Applied Biosystems, Carlsbad, CA), as per manufacturer's protocol. The 25 µl reactions allowed for the addition of 15 µl template; the maximum amount used was 0.5 ng DNA in a STR amplification, though most samples had much less than this in the PCR. Separation of PCR products was accomplished by capillary electrophoresis (CE) in a 3500*xl* Genetic Analyzer followed by STR data analysis using the GeneMapper®ID-X v1.4 software program (Applied Biosystems, Carlsbad, CA) or data analysis using TrueAllele® Casework probabilistic modeling system (Cybergenetics, Pittsburgh, PA).

As part of our initial method development we also tested whether direct amplification and STR profiling of the sorted cell populations with the Powerplex Fusion system compared with results obtained from DNA IQ™ extraction. Direct amplification was performed according to the manufacturer's protocol with the following modification: 15 µl PunchSolution™ Reagent was added to a PCR tube containing the pelleted cell sample or reagent blank, mixed by pipetting, capped, and incubated at 70 °C for 30 min. The entire sample was then subjected to PCR amplification. Results indicated no clear differences in the number of alleles detected across either method (comparison tables shown in Table S1). All results reported in this study were obtained using DNA IQ™ method for extraction of DNA from unsorted mixture samples, contributor reference samples, and sorted cell fraction P2 and P3.

Qualitative (analyst) assessment of STR profiles followed Virginia Department of Forensic Science (VDFS) procedures for calling alleles, examination of controls and identification of artifacts in samples. For mixture samples, allele assignment to contributors was based on comparison to known donor reference profiles. Alleles were noted as either unique to a donor, shared with at least one other donor, or non-donor (not attributable to any of the contributors of the sample). In a casework setting, qualitative approaches alone would not utilize all of the data present within an STR profile, underscoring the need for quantitative interpretation protocols such as TA. Thus, we used both qualitative and quantitative analyses of mixtures for this study. Quantitative assessment of selected STR profiles was performed using TrueAllele® Casework software [5,8]. This probabilistic modeling system uses all of

the peak height and position data from an electropherogram to develop most likely explanations for the profile by use of Markov chain Monte Carlo (MCMC) sampling of the data. The TrueAllele® Casework (TA) mixture deconvolution process is performed in the absence of any reference profiles unless a reference is "assumed". No references were assumed for this study. There is no drop-in or drop-out factor calculated or needed for the TA analysis process. Instead, the allele data, in the form of peaks, is modeled *de novo* for each electropherogram. Every possible allele pair combination is tested and the probability assessed to explain that mixture profile. After the mixture deconvolution process is complete, then comparisons, in the form of likelihood ratios, are performed for all reference profiles of interest. Moreover, the TA process requires a minimum of two reproducible independent TA analyses of the STR data, thus if a value brackets zero, small positive log(LR) for one run and small negative log(LR) for the other, it will also be interpreted as inconclusive.

The hypothesis utilized in this study for all mixtures was as follows: the LR hypothesis (Hp) is that a person contributed their DNA to the mixture, along with N-1 unknown contributors. The alternative (Hd) is that the mixture contains N unknown contributors. Qualitative and quantitative assessments of blood samples were compared for concurrence of results.

3. Results and discussion

3.1. Blood mixture samples

Blood from five different contributors was used to prepare mixture samples derived from two, three, four or five of those donors in specified ratios (Table 1). White blood cells from each of these mixture samples were labeled with HLA-A*02 antibody and sorted by FACS to the P2 or P3 fractions corresponding to cell populations that bound to the antibody probe and cell populations that did not bind to the probe, i.e., A*02 positive and A*02 negative phenotypes, respectively. The fluorescence histograms and sorting gates for the two contributor mixtures are shown in Fig. 1, while the three, four, and five contributor fluorescence histograms and sorting gates are shown in Fig. 2.

STR profiles were generated from each sorted cell population and compared to the reference profiles of the contributors for that mixture sample. For three, four, and five contributor mixtures, alleles unique to each contributor are color-coded in the genotype table for ease of visualization. Each donor was assigned a color: donor 103 = gold, 104 = purple, 105 = red, 106 = green, 107 = blue. Within each subsample profile an allele unique to a donor was marked with that donor's color (Tables 4,6,8,10), otherwise uncolored boxes indicate that allele was shared by more than one donor in the mixture. All mixture samples and sorted cell populations were qualitatively analyzed in this manner.

3.2. Blood mixture samples – two contributor mixtures

Two separate, two contributor mixtures containing an A*02 positive and an A*02 negative contributor were created in 1:1 ratios. The fluorescence histogram of the first cell mixture (contributors 93 and 94) after antibody hybridization shows two distinct peaks consistent with the presence of an A*02 positive and an A*02 negative contributor (Fig. 1 left panel). DNA profiling of the unsorted mixture (Table 2) showed full STR profiles for both donors. After sorting, the P2 sorted fraction (A*02 positive) showed a complete, single source profile for donor 94. There were no alleles from 93 detected in this fraction (Table 2). The P3 sorted fraction (A*02 negative) showed a full profile for the negative donor, 94, with only five minor alleles consistent with 93 detected. The peak height ratio of major to minor contributor ranged between 8:1 to 10:1.

A second mixture composed of donors 95 and 96 showed similar results (Table 3). Although the fluorescence histogram showed two distinct peaks consistent with an A*02 positive and an A*02 negative contributor, cell populations exhibited more apparent overlap with less distinct differences in peak fluorescent intensity compared to the previous mixture histogram (Fig. 1, right). Complete STR profiles for both

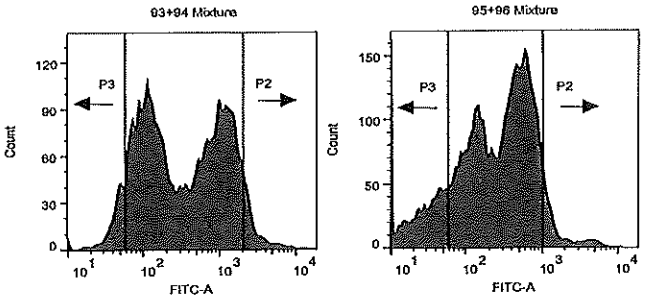

Fig. 1. Fluorescence histograms and sorting gates for 93 + 94 and 95:96 two contributor mixtures. HLA-A*02-labeled cells were sorted into the P2 fraction, and HLA-A*02-unlabeled cells were sorted into the P3 fraction.

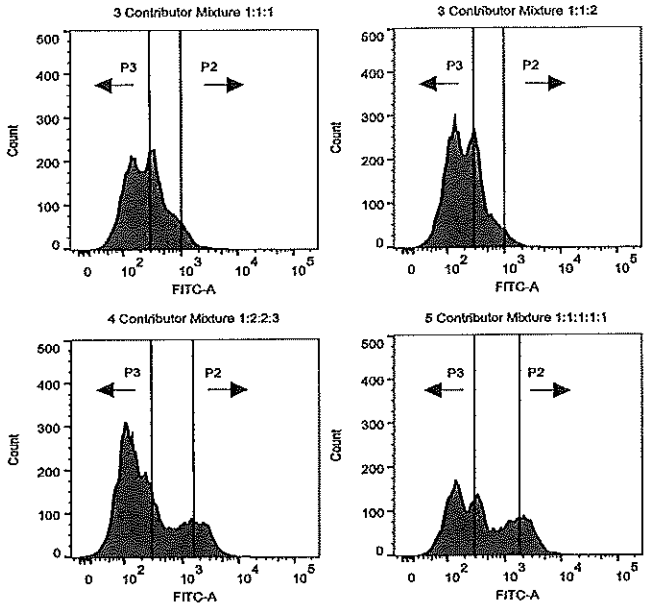

Fig. 2. Fluorescence histograms and sorting gates for the three, four, and five contributor mixtures. HLA-A*02-labeled cells were sorted into the P2 fraction, and HLA-A*02-unlabeled cells were sorted into the P3 fraction.

Table 2
STR profiles from two-person mixture (Donors 93, 94).

	94 Reference (−)	93 Reference (+)	93 + 94 UnSorted [1]	P2 Sorted [1]	P3 Sorted [1]
D8S1179	13,15	10,13	10,13,15	10,13	13,15
D21S11	28,32.2	28,31	28,31,32.2	28,31	28,32.2
D7S820	12	10,11	10,11,12	10,11	12
CSF1PO	10,11	10,12	10,11,12	10,12	10,11
D3S1358	16,17	16,17	16,17	16,17	16,17
TH01	6,7	7,8	6,7,8	7,8	6,7
D13S317	11,12	8,12	8,11,12	8,12	11,12
D16S539	11,12	9,13	9,11,12,13	9,13	(9),11,12
D2S1338	20,25	19,24	19,20,24,25	19,24	(19),20,25
D19S433	13,15	12.2,15.2	12.2,13,15,15.2	12.2,15.2	13,15
VWA	17	15,16	15,16,17	15,16	17
TPOX	8,10	8,9	8,9,10	8,9	8, (9),10
D18S51	15,17	12,17	12,15,17	12,17	15,17
AMEL	XY	XY	XY	XY	XY
D5S818	11,13	8,12	8,11,12,13	8,12	11, (12),13
FGA	23	21	21,23	21	(21),23

[1] Minor peaks are shown in parentheses.

Table 3
STR profiles from two-person mixture (Donors 95, 96).

	95 Reference (A*02−)	96 Reference (A*02+)	95 + 96 UnSorted [1]	P2 Sorted [1]	P3 Sorted [1]
D8S1179	14,16	14,15	14,15,16	14,15	14,16
D21S11	28	28,29	28,29	28,29	28
D7S820	11	8,10	8,10,11	8,10	11
CSF1PO	8,10	10,12	8,10,12	10,12	8,10
D3S1358	15,16	15,16	15,16	15,16	15,16
TH01	8,9	6,7	6,7,8,9	6,7,(8)	8,9
D13S317	10,13	11,12	10,11,12,13	11,12,(13)	10,13
D16S539	11	8,10	8,10,11	8,10,(11)	11
D2S1338	16,20	22,25	16,20,22,25	(16), (20),22,25	16,20
D19S433	12,14	13.2,18.2	12,13.2,14,18.2	13.2, (14),18.2	12,14
VWA	15,18	15,17	15,17,18	15,17	15,18
TPOX	8,11	8,11	8,11	8,11	8,11
D18S51	15,17	16,23	15,16,17,23	16,23	15,17
AMEL	XX	XY	XY	XY	XX
D5S818	11,12	11,12	11,12	11,12	11,12
FGA	22,24	22,23	22,23,24	22,23,(24)	22,24

[1] Minor peaks are shown in parentheses.

donors were detected in the unsorted mixture. The P2 sorted fraction contained a full profile for the positive donor, 96, with seven minor alleles detected from the negative donor, 95. The peak height ratio of major to minor contributor ranged in this mixture between 8:1 to 10:1. The P3 sorted fraction gave a complete, single source profile for the negative donor. No alleles from the positive donor were detected in this fraction (Table 3).

Results from the two-person mixtures indicate that antibody hybridization can be used to selectively label and sort contributor cell populations in a dried blood sample. Easily interpretable STR profiles consistent with each contributor were obtained from the two sorted cell fractions with only minor contributions from the non-target contributor. The decreased separation between A*02 positive and A*02 negative populations compared to earlier studies (i.e., (3)) may be due to increases in autofluorescence after drying as suggested previously [4] or due to increases in non-specific probe interactions due to degradation of cell targets after drying. Alternatively, differences in the efficiency of antibody hybridization may be donor-specific depending the presence of cross-reactive HLA alleles, i.e., non-A*02 antigens binding to A*02 antibody probe [14].

3.3. Blood mixture samples – three contributor mixtures

Next, three donor samples (105, 106, and 107) were used to create two separate, three contributor blood mixtures in ratios of 1:1:2 and 1:1:1. Donor 106 was HLA-A*02 positive whereas donors 105 and 107 were HLA-A*02 negative (Table 1). Therefore, in both mixtures the P2 fraction should have been enriched in donor 106 whereas the P3 fraction should have been enriched in cells from donors 105 and 107. For the 1:1:2 mixture, STR profiles from the unsorted mixture yielded full profiles for all three contributors and profiles from the P2 fraction primarily contained alleles consistent with donor 106 and with only two alleles from donor 107 and two alleles from donor 105 detected (Table 4). The STR profile from the P3 fraction was enriched for donors 105 and 107, with six alleles from donor 106 detected (Table 4).

Quantitative assessment by TA confirmed the qualitative results for the three contributor mixture sample (1:1:2). TA log(LR) values for the unsorted subsample were within 100-fold of each other, ranging from 9.4714 to 11.4591 (Table 5), which is equivalent to likelihood ratios of 2.9 billion and 287 billion, respectively. This indicates that it is 2.9 billion to 287 billion times more probable to observe the obtained DNA results if the person of interest contributed their DNA to the mixture,

Table 4
Genotype table for the three contributor (1:1:2) blood mixture.(For interpretation of the references to colour in this table legend, the reader is referred to the web version of this article.)

	Unsorted Mixture 1:1:2	Sorted P2 (A*02+)	Sorted P3 (A*02-)
Marker			
AMEL	X Y	X Y	X Y
D3S1358	14 15 17	15 17	14 15 17
D1S1656	11 13 15.3 17 18.3	13 17.3 18.3	11 13 15.3 17.3
D2S441	10 11 11.3	10	10 11 11.3
D10S1248	13 14	13	13 14
D13S317	11 12 13	12	11 13
Penta E	5 7 11 12 15	11	5 7 11 12 15
D16S539	11 12 13	12 13	11 12 13
D18S51	13 14 16 17	14 16 17	13 16 17
D2S1338	17 19 20 23 24	17 20	19 23 24
CSF1PO	10 11 12	11	10 11 12
Penta D	2.2 9 10 13 14	9 13	2.2 10 13 14
TH01	6 7 9	6 7	6 7 9
vWA	15 16 18 19 20	15 20	15 16 18 19
D21S11	28 30 31 33.2	28 31	28 30 33.2
D7S820	10 11 12 13	11	10 11 12
D5S818	11 12 13	11 13	11 12 13
TPOX	8 9 11 12	9 11	8 11 12
DYS391	11	11	11
D8S1179	13 14 15	13 14 15	13 14 15
D12S391	17 18 19 20 22 23	17 23	17 18 19 20 22
D19S433	12 13 14	12 13	12 13
FGA	19 20 21 23 24 25	21 23	19 20 23 24 25
D22S1045	11 15 16	16	11 15 16

Red = 105, Green = 106, Blue = 107.

Table 5
TrueAllele® Casework analysis for the three contributor (1:1:2) blood mixture sample.(For interpretation of the references to colour in this table legend, the reader is referred to the web version of this article.)

	log Likelihood Ratio		
Contributor[1]	Unsorted	Sorted P2 (A*02+)	Sorted P3 (A*02-)
105 (HLA-A02-)	9.7462	-16.5399	11.5894
106 (HLA-A02+)	11.4591	13.0249	-15.7665
107 (HLA-A02-)	9.4714	-11.9631	20.5656

[1]Donor colors correspond to genotype charts in Table 4.

Table 6
Genotype table for the three contributor (1:1:1) blood mixture.(For interpretation of the references to colour in this table legend, the reader is referred to the web version of this article.)

	Unsorted Mixture 1:1:1						Sorted P2 (A*02+)		
Marker									
AMEL	X	Y					X	Y	
D3S1358	14	15	17				15		
D1S1656	11	13	15.3	17.3	18.3		13	17.3	18.3
D2S441	10	11	11.3				10		
D10S1248	13	14							
D13S317	11	12	13				12		
Penta E	5	9	11	12	13		12		
D16S539	11	12	13				11	13	
D18S51	12	14	16	17			14	17	
D2S1338	17	19	20	23	24		17	20	
CSF1PO	10	11	12				12		
Penta D	2.2	9	10	13	14		9		
TH01	6	7	9						
vWA	15	16	18	19	20		20		
D21S11	28	30	31	31.2					
D7S820	10	11	12	13					
D5S818	11	12	13				11	13	
TPOX	8	9	11	12			11		
DYS391	11						11		
D8S1179	12	13	14	15			14	15	
D12S391	17	18	19	20	22	23	23		
D19S433	12	13	14				13		
FGA	19	20	21	23	24	25	23		
D22S1045	11	15	16						

Red = 105, Green = 106, Blue = 107.

Table 7
TrueAllele® Casework analysis for the three contributor (1:1:1) blood mixture sample.(For interpretation of the references to colour in this table legend, the reader is referred to the web version of this article.)

	log Likelihood Ratio	
Contributor[1]	Unsorted	Sorted P2 (A*02+)[2]
105 (HLA-A02-)	13.1799	-15.3381
106 (HLA-A02+)	14.5489	5.1996
107 (HLA-A02-)	17.8216	-1.7661

[1]Donor colors correspond to genotype charts in Table 6.
[2]DNA was not detected in the P3 fraction for this mixture.

along with N-1 unknown contributors, than if the mixture contains N unknown contributors. TA analysis of the sorted cell populations provided quantitative support that donor 106 was enriched in the sorted P2 fraction and donors 105 and 107 were enriched in the sorted P3 fraction. Specifically, the sorted P2 fraction subsample yielded a log(LR) of 13.0249 for contributor 106, an enrichment of almost 100-fold greater from the unsorted subsample. Concurrently the log(LR) values for contributors 105 and 107 in the sorted P2 subsample were -16.5399 and -11.9631, suggesting that they were excluded from the P2 cell population (Table 5). TA analysis of the sorted P3 subsample yielded a negative log(LR) value for donor 106, which indicated that this contributor was excluded from the P3 subsample. Donor 105 had a log(LR) value of 11.5894, an almost 100-fold increase from the unsorted log (LR) of 9.7462. Donor 107 displayed a log(LR) value of 20.5656 in the sorted P3 subsample, an increase of more than 11 orders of magnitude from the unsorted subsample (Table 5).

Although DNA was not recovered from the P3 fraction of the 1:1:1 mixture, STR profiling results from the unsorted mixture and the P2 fraction suggested efficient separation of the A*02 positive contributor from the mixture. Specifically, full STR profiles for each of the three contributors were detected in the unsorted fraction whereas a full profile for only donor 106 was detected in the P2 fraction (Table 6). Only one allele from a non-target contributor (107) was detected in this fraction. TA analysis indicated that there was only statistical support for

Table 8
Genotype table for the four contributor (1:2:2:3) blood mixture.(For interpretation of the references to colour in this table legend, the reader is referred to the web version of this article.)

Marker	Unsorted Mixture 1:2:2:3						Sorted P2 (A*02+)						Sorted P3 (A*02-)					
AMEL	X	Y					X	Y					X	Y				
D3S1358	14	15	16	17			14	15	16	17			14	15	16	17		
D1S1656	11		13	15.3	17	18.3	11		13	15.3	17.3		11		13	17.3	18.3	
D2S441	10	11	11.3				10	11	11.3				10	11	11.3			
D10S1248	13	14	15				13	14	15				13	14	15			
D13S317	11	12	13				11	12	13				11	12	13			
Penta E	5	11	12	14		19	5	11	12	14	19		5	11	12	14		19
D16S539		11	12	13				11	12	13				11	12	13		
D18S51		14	15	16	17			14	15	16	17			14	16	17		
D2S1338	17	19	20				17	19	20				17	19				
CSF1PO	10	11	12				10	11	12				10	11	12			
Penta D	2.2	9	10		13		2.2	9	10	13			2.2	9	10		13	
TH01	6	7	9	9.3			6	7	9	9.3			6	7	9			
vWA	15	16		18	19	20	15	16		20			15	16		18	20	
D21S11	28	29	30	31			29	30	31				28	29	30	31		
D7S820		10	11	12	13		10	11	12					10	11	12	13	
D5S818	11	12	13				11	12	13				11	12	13			
TPOX	8	9	11				9	11					8	9	11			
DYS391	10	11					10						10	11				
D8S1179	13	14	15				13	14	15				13	14	15			
D12S391	17	18	20	22	23		17	18	20	22	23		17	18	20	22	23	
D19S433	12	13	14	15			12	13	14	15			12	13	14	15		
FGA	21	22.2	23	24	25		21	22.2	23	24			21	22.2	23	24	25	
D22S1045	11	15	16	17			15	16	17				11	15	16			

Yellow = 103; Purple = 104; Green = 106; Blue = 107.

Table 9
TrueAllele® Casework analysis for four contributor (1:2:2:3) blood mixture sample.(For interpretation of the references to colour in this table legend, the reader is referred to the web version of this article.)

	log Likelihood Ratio		
Contributor[1]	Unsorted	Sorted P2 (A*02+)	Sorted P3 (A*02-)
103 (HLA-A02+)	5.6952	25.8097	-0.4071
	9.3661	1.9489	13.8653
106 (HLA-A02+)	10.2259	3.902	4.5862
107 (HLA-A02-)	6.3227	4.6193	10.6459

[1]Donor colors correspond to genotype charts in Table 8.

donor 106 in the P2 fraction (5.1996 106 compared to -1.7661 for 107 and -15.338 for 105, Table 7).

3.4. Blood mixture samples – four contributor mixture

The 1:2:2:3 four contributor blood mixture sample was prepared with donors 103, 104, 106, and 107. STR profiles from the unsorted subsample yielded full profiles for all four contributors (Table 8). Based on respective HLA phenotypes, P2 should have been enriched in donors 103 and 106 and P3 should have been enriched in donors 104 and 107. The actual STR profile from the P2 fraction was enriched for donor 103, as seen by the frequency of gold colored alleles in the genotype chart (Table 8, center). The P2 fraction shows few alleles from donor 106, compared to what we would expect given the dominance of their alleles in the P2 fraction of the three contributor mixtures. The STR profile resulting from the P3 fraction shows alleles consistent with donors 104 and 107, representing all alleles for both of those contributors. All alleles consistent with 106, except for one allele at D2S1338 are also present, however a qualitative analysis does not utilize much if any allele peak height information and mixture weight assessments and thus does not always provide a complete picture of the data.

Probabilistic modeling showed evidence of all four contributors in the unsorted mixture with LR values of ~5.7, 6.3, 9.3, and 10.2 for donors 103, 107, 104, and 106 respectively (Table 9). After sorting, the P2 fraction showed significant enrichment for donor 103 (25.8097) and the P3 fraction showed significant enrichment for donors 104 and 107 (13.8653, 10.6459 respectively). There was only limited statistical association of donor 106 in either sorted cell fractions (3.902 in P2 and 4.5862 in P3). Overall, TA analysis provided quantitative support for one of the A*02 positive contributors and both A*02 negative contributors in the corresponding sorted cell fractions. Although a few unique alleles for donor 106 were detected in the unsorted mixture profile as well as the sorted P2 and P3 fractions (Table 8), the lower statistical support for 106 from TA analysis suggests proportionally fewer cells were sorted into either P2 or P3 fractions. This may be due to incorrect partitioning of donor 106 cells into the P3 fraction from inefficient antibody hybridization. We note that poor detection of alleles from donor 106 was observed in multiple cell mixtures for this study (1:2:2:3 and 1:1:1:1:1 shown below). Direct comparison of hybridized cell populations from the donors 103 and 106 (both A*02

Table 10
Genotype table for the five contributor (1:1:1:1:1) blood mixture.(For interpretation of the references to colour in this table legend, the reader is referred to the web version of this article.)

	Unsorted Mixture 1:1:1:1:1										Sorted P2 (A*02+)										Sorted P3 (A*02-)									
Marker																														
AMEL	X	Y									X	Y									X	Y								
D3S1358	14	15	16	17							16	17									14	15	17							
D1S1656	11		13	15.3	17.3	18.3					11	15.3									11		13	15.3	17.3					
D2S441	10	11	11.3								11	11.3									10	11	11.3							
D10S1248	13	14	15								13	15									13	14	15							
D13S317	11	12	13								12										11	12	13							
Penta E	5		11	12		14		19			14	19									5		11	12			19			
D16S539		11	12	13							11	12	13									11	12							
D18S51	12	14	15	16	17						14	15	16	17							12	16	17							
D2S1338	17	19	20		24						17	19									17	19								
CSF1PO	10	11	12								10	11	12								10	11	12							
Penta D	2.2	9	10		13	14					9	13	14								2.2	9	10		13	14				
TH01	6	7	9	9.3							6	9.3									6	7	9							
vWA	15	16		18	19	20					15	19									15	16		18	19					
D21S11	28	29	30	31							29	31									28	30	31							
D7S820		10	11	12	13						10											10	11	12						
D5S818	11	12	13								11	12	13								11	12	13							
TPOX	8	9	11	12							9	11									8	11	12							
DYS391	10	11									10										11									
D8S1179	12	13	14	15							13	14	15								12	13	14	15						
D12S391	17	18	19	20	22	23					17	22									18	19	20	22						
D19S433	12	13	14	15							14	15									12	13								
FGA	19	20	21	22.2	23	24	25				19	22.2	23	24							19	21	24	25						
D22S1045	11	15	16	17							16	17									11	15	16							

Yellow = 103; Purple = 104; Red = 105; Green = 106; Blue = 107.

Table 11
TrueAllele® Casework analysis for five contributor (1:1:1:1:1) blood mixture sample.(For interpretation of the references to colour in this table legend, the reader is referred to the web version of this article.)

	log Likelihood Ratio		
Contributor[1]	Unsorted	Sorted P2 (A*02+)	Sorted P3 (A*02-)
103 (HLA-A02+)	7.7709	28.0754	-3.7186
	7.7705	-10.3345	19.1138
105 (HLA-A02-)	8.4417	-7.6818	9.1347
106 (HLA-A02+)	7.9141	-7.8931	-12.6020
107 (HLA-A02-)	3.4128	-16.3560	9.4315

[1]Donor colors correspond to genotype charts in Table 10.

positive) after drying indicates that donor 103 cells have stronger interaction with the probe as evidenced by higher proportion of cells above 10 [3] RFU (Figure S2). Additionally, mixtures in which donor 106 is the only A*02 positive contributor exhibit lower fluorescence intensities in the P2 subpopulation compared to mixtures where donor 103 is present (e.g., P2 populations in top two histograms versus bottom two histograms in Fig. 2), further suggesting that this is a contributor-specific trend.

3.5. Blood mixture samples – five contributor mixture

The five contributor 1:1:1:1:1 blood mixture sample was composed of donors 103, 104, 105, 106, and 107. All alleles consistent with the five donors were observed in the unsorted subsample (Table 10). In a forensics laboratory this would be a very challenging mixture, not only due to the number of contributors but also because all were present in equal measure. In many, if not most forensic laboratories, the mixture would be deemed uninterpretable due to its complexity and potential information would be lost.

Donors 103 and 106 were HLA-A*02 positive and are expected to sort into the P2 fraction whereas donors 104, 105 and 107 were HLA-A*02 negative and are expected to sort into the P3 fraction. Qualitatively, the STR profile generated from the sorted P2 fraction was enriched for donor 103, with 10 unique alleles detected from this contributor compared to three unique alleles detected from contributors 105 and 106 (Table 10). The STR profile generated from the sorted P3 fraction showed the highest number of unique alleles for donors 104 (= 8), 105 (= 10), and 107 (= 9), with only three alleles detected that were uniquely attributable to donors 103 or 106 (Table 10). Qualitative assessment determined that the sorted P2 subsample yielded all alleles for donor 103, and the sorted P3 subsample generated all alleles for donors 104 and 107 and nearly all for 105. The limited number of unique alleles from donor 106 detected in the P2 fraction are consistent with results from the 1:1:2 mixture and could indicate less efficient antibody hybridization to this contributor cell population and subsequent sorting into the A*02 negative fraction

(discussed further below). Additionally, physical adhesion or clumping of target cells to non-target cells has been observed in previous flow cytometry studies [3] and could have contributed to non-specific sorting, and allelic drop-out in this experiment.

Quantitative assessment of the five contributor sample was performed using TA (Table 11). The unsorted subsample included all five donors, with log(LR) values ranging between 3.4128-8.4417. TA analysis of the sorted P2 subsample yielded log(LR) of 28.0754 for donor 103, a value that is comparable to a single source sample [15], and negative values for the other four contributors. The sorted P3 subsample yielded log(LR) of 19.1138 for donor 104, an increase of nearly 12 orders of magnitude. While donor 105 produced equivalent values for unsorted and sorted fractions, the log(LR) for donor 107 increased by 6 (a million times more likely increase). Donors 103 and 106 showed LR values of -3.7186 and -12.6020 respectively suggesting that they were excluded from the sorted P3 subsample. The TA results provided quantitative confirmation that donor 103 was enriched in the sorted P2 fraction, donors 104 and 107 were enriched in the sorted P3 fraction while donor 105 stayed the same, and that donor 106 was not detected after the cell sorting process.

4. Conclusions

The data presented here suggests that antibody probes combined with FACS can be used for the front-end separation of contributor cell populations in two-person dried blood mixture samples to generate single source STR profiles. Further, for mixtures containing three, four, or five individuals, binary sorts based on the presence or absence of an HLA allele can be combined with probabilistic modeling procedures to enhance the interpretation of complex mixture samples. Results from mixtures containing three or more individuals may potentially be further improved by combining different antibody probes in the initial hybridization steps to enhance discrimination of cell populations during FACS and/or sorting cell populations into more than two fractions (i.e., non-binary sort) depending on the nature of the fluorescence histogram and the initial resolution of contributor cell populations with the mixture sample. Alternative antibody probes may be particularly useful for labelling contributor cell populations that exhibit decreased separation efficiency with a given probe (e.g., donor 106 with A*02 probe). With this, it may be necessary to systematically investigate binding efficiencies of specific antibody probes against dried cell populations containing a range of subtypes of the target allele (e.g., subtypes of A*02 described in ([16])) as well as cell populations with non-target antigens within the same cross-reactive group as the target allele [14,17].

Future studies can also make effort to collect and profile cells from the discarded fraction of the FACS instrument that result from sorting errors or events falling outside the initial gating parameters for target cells. For some complex mixtures retaining this fraction may help detect certain contributor cell populations that are incorrectly sorted and also would be a generally advantageous practice for degraded and/or low template samples. Although these results suggest that antibody based cell labelling and FACS separation may be used on dried/compromised samples, we acknowledge that as the extent of sample degradation increases (i.e., dried for > 24 h), decomposition of antigen targets and/or autofluorescence may present more significant obstacles. Applying this workflow to the full range of sample types and conditions encountered in forensic casework may require more robust probe targets or alternative autofluorescence-based signatures that can be detected in aged samples [18].

Grant information

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.fsigen.2018.07.004.

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EXHIBIT 9

SCIENTIFIC ADVISORY COMMITTEE

October 15, 2013 9:00 am
Department of Forensic Science
Central Laboratory Training Room 1
Proposed Agenda

I. Call to Order – *Committee Chair Jami St. Clair*

- Welcome New SAC Members

II. Approval of Agenda

III. Approval of Minutes of May 14, 2013 meeting

IV. Chair's Report, if any

V. DFS Director's Report – *Director Linda C. Jackson*

- DFS Update
  - Facilities
  - Workload/Backlogs
  - Grants
  - Resources and Budget Outlook
  - Laboratory News/Updates
    - Staffing and Instrumentation
    - Redesigned DFS Website
    - Post-Conviction DNA Testing Program
    - Other updates, if any

VI. Old Business

- True Allele: DFS Validation Studies and Case Experience -- *Brad Jenkins, DFS Biology Program Manager*
- Report of DNA/Biology Subcommittee on True Allele – Consideration of Recommendation by SAC -- *Brad Jenkins*
- Latent Prints Manual / Mideo Implementation – *Sabrina Cillesson, DFS Physical Evidence Program Manager*
- Trace Evidence Update – *Scott Maye, DFS Chemistry Program Manager*

VII. New Business

- Outlook for 2014 Legislation – *Gail Jaspen, DFS Chief Deputy Director*

VIII. Public Comment, if any

IX. Confirm future meeting dates

X. Adjourn

EXHIBIT 10

FORENSIC SCIENCE BOARD

Wednesday, October 16, 2013 9:00 am
Central Laboratory, Classroom 1
Draft Agenda

- Call to Order and Welcome – *Board Chair Jo Ann Given*
- Adoption of Agenda
- Approval of Draft Minutes of August 7, 2013 meeting
- Board Chair's Report, if any
- DFS Director's Report – *Director Linda Jackson*
  - State of the Agency
    - Facilities
    - Resources – Instrumentation / Budget Overview
    - Workloads / Backlogs
    - Customer Communication
    - New DFS Website
    - Grants
    - Implications of Federal Government Shutdown (TENTATIVE)
- Scientific Advisory Committee Report and Recommendations – *SAC Chair Jami St. Clair*
- Old Business
  - Status of Proposed Regulatory Amendments – *Department Counsel Stephanie Merritt*
  - Status of the Post-Conviction DNA Testing Program and Notification Project Agency
    - Notification Subcommittee Report – *Subcommittee Chair Kristen Howard*
    - Testing Program Update – *DFS Chief Deputy Director Gail Jaspen*
  - Western Region SAVVY Expo – *Gail Jaspen*
- New Business
  - Reaccreditation – *DFS Director of Technical Services Alka Lohmann*
  - Review and Adoption of Proposed Annual Report of the Board Pursuant to VA Code § 9.1-1110 – *Gail Jaspen*
  - Outlook for 2014 Legislation – *Gail Jaspen*
- Public Comment, if any
- Confirm future meeting dates
- Adjourn

EXHIBIT 11

Welcome to the world of TrueAllele® Cloud

TrueAllele Casework objectively interprets complex DNA evidence. Starting from laboratory data, TrueAllele quickly separates mixtures to produce accurate match statistics. The technology resolves degraded and touch DNA, and mixtures with many contributors or relatives. TrueAllele routinely makes DNA identifications for police, prosecutors, defense lawyers, crime labs, and actual innocence.

The TrueAllele Cloud lets anyone test DNA evidence, without having to purchase a system. Forensic scientists and students can learn about TrueAllele on the Cloud. Crime laboratories use the TrueAllele Cloud for validation, training, and extra computer capacity. Defense lawyers and experts can confirm TrueAllele case reports.

If you are interested in testing DNA mixtures your own data using TrueAllele on the Cloud, please contact Cybergenetics at data@cybgen.com or call 412-683-3004 for more information.

EXHIBIT 12

TrueAllele® Methods: Statistical Model

System 3, Version 25
September 2008 model

Mark Perlin, PhD, MD, PhD
Cybergenetics, Pittsburgh, PA
8 March 2016

Overview

This document provides scientific background and mathematical formulas for statistical modeling in the TrueAllele system. The document complements previously published descriptions of the hierarchical Bayesian model for genotype separation.

Data

Short tandem repeat (STR) data originate as charge-coupled device (CCD) camera counts that are collected on a genetic analyzer from fluorescently end-labeled DNA fragments as they are separated by size via gel electrophoresis. These multi-spectral CCD signals are isolated by their fluorescent dyes using a color separation matrix to form dye-specific signals via matrix inversion.

Signal analysis identifies a DNA data peak corresponding to a particular DNA fragment. Using allelic and internal size ladders, the analysis determines a DNA peak's *size* (bp) and allele length (repeats), as well as the DNA *quantity* measured in relative fluorescent units (rfu). A data vector records the DNA quantity (including zero) for every fragment size.

We model the quantitative data at STR locus l (of L loci) using several variables. Data vector $\mathbf{d}_l$ forms a pattern that maps DNA product lengths into their observed quantitative peak heights.

We linearly model the data vector $\mathbf{d}_l$ using a truncated ($\geq \mathbf{0}$) multivariate normal distribution N_+ of the mean vector μ_l and covariance matrix Σ_l as

$$\mathbf{d}_l \sim N_+(\mu_l, \Sigma_l)$$

We write the peak data covariance matrix Σ_l as

$$\Sigma_l = \sigma^2 \cdot V_l + \tau^2$$

where σ^2 is amplification dispersion, τ^2 is detection variation, and V_l is a diagonal matrix $diag(\mathbf{d}_l)$ of peak heights.

Data Usage

TrueAllele determines statistical parameters directly from the data, mining DNA evidence for statistical information. The fully Bayesian system does not require calibration (i.e., setting parameters from historical laboratory data unrelated to evidence data).

TrueAllele inputs and uses all the data. There are no thresholds, since uncertainty is determined statistically from the data. There is an optional rfu cutoff, usually set at ten rfu (within the background noise), well below allelic peak events. Should alleles be observed below this level, the cutoff can be lowered or turned off.

Allele dropout occurs when alleles that are present in the genotypes do not appear in the data signal. Bayesian modeling accounts for all genotype possibilities, whether or not component alleles manifest themselves in the genotyping data. TrueAllele assesses allele dropout through a likelihood function, assigning lower probabilities to genotype proposals that have less support in the data. TrueAllele addresses allele drop-in events in a similar way. There are no explicit drop parameters – Bayes theorem with an informative likelihood function addresses data drop phenomena.

Using all the data is thorough and preserves identification information. Eliminating human data decisions (choosing loci, peaks, artifacts) removes human bias from the

interpretation process. Users cannot "control their data." The user supplies the data, makes few assumptions (number of contributors, sampling time, degradation option), and the rest is done automatically by the computer system.

TrueAllele can answer questions about different data combinations. Data from multiple items or amplifications can be used in a joint genotype analysis. Known genotypes (e.g., a victim present in a mixture, ascertained by case context or match statistics) can help reduce problem complexity.

A comparison genotype (e.g., a suspect) cannot be part of interpreting evidence. The computer does not know the "answer" when it separates genotype from evidence. A match comparison is only made afterwards. Guaranteeing that genotype inference is entirely separate from match statistic calculation helps ensure process objectivity.

Mass

DNA is packaged in the cell nucleus. This cell packaging is opened when DNA is extracted from a biological sample and made available for laboratory analysis. The mass, or number of intact DNA molecules examined in a test tube, is modeled as a normal random variable.

The total DNA quantity at locus l is given by mass parameter m_l. The locus mass m_l prior is a (nonnegative) truncated normal distribution on feasible total peak rfu values.

$$m_l \sim N_+\left(5000, 5000^2\right)$$

Genotype

Individuals inherit DNA from two parents. Therefore, at a given genetic locus on an autosomal chromosome, a cell has two alleles (STR length variants), one from each parent. This pair of alleles is called a *genotype*. A genotype is represented as a vector of all possible allele sizes, with each vector entry containing a number of alleles at that particular size.

With K contributors to the data, we represent the k^{th} contributor genotype parameter at locus l as a vector $\mathbf{g}_{k,l}$, where the DNA length entries contain allele counts

that sum to 1. A heterozygote genotype vector $\mathbf{g}_{k,l}$ contains two 1/2 entries, while a homozygote has a single 1 entry; all other vector entries are 0.

The genotype *prior* probability $\Pr\{\mathbf{g}_{k,l} = x\}$ at allele pair $x = [i\ j]$ is a product of population allele frequencies $\{f_i\}$.

$$\mathbf{g}_{k,l} \sim \begin{cases} f_i^2, & i = j \\ 2 f_i f_j, & i \neq j \end{cases}$$

TrueAllele's *likelihood* function assesses a genotype candidate value to determine how well it explains the observed data. The likelihood is larger when the quantitative data is better accounted for by a predicted peak height pattern based on the allele pair value. For the i^{th} data observation $d_{l,i}$ at locus l, the likelihood function for a genotype $\mathbf{g}_{k,l}$ is the probability $\Pr\{d_{l,i} \mid \mathbf{g}_{k,l} = x, \ldots\}$ of the data conditioned on genotype value x, where "..." denotes the other model variable values, given by the data distribution $\mathbf{d}_l \sim N_+(\mu_l, \Sigma_l)$.

Combining the prior genotype probability together with I independent genetic data observations, we can compute the *posterior* genotype probability using Bayes theorem as the product of prior probability and joint likelihood functions. The probability mass function (pmf) $q(x)$ of genotype $\mathbf{g}_{k,l}$ is the joint probability distribution

$$\Pr\{\mathbf{g}_{k,l} = x \mid d_{l,1}, d_{l,2}, \ldots, d_{l,i}, \ldots\} \propto \Pr\{\mathbf{g}_{k,l} = x\} \cdot \prod_{i=1}^{I} \Pr\{d_{l,i} \mid \mathbf{g}_{k,l} = x, \ldots\}$$

over all the relevant random variables.

Mixture Weight

A mixture contains DNA from two or more people. The relative amount of DNA from a person contained in the mixture is a *mixture weight* value between zero and one. The sum of the mixture weights over all the people contributing the mixture is one.

The mixture weight parameter at locus l is a vector $\mathbf{w}_l$ whose K contributor components sum to 1, so that $\sum_{k=1}^{K} w_{k,l} = 1$. A hierarchical model of mixture weight at every

locus provides a better fit to the data. We therefore draw each individual locus weight $\mathbf{w}_l$ as a hierarchical prior from a common DNA template mixture weight $\mathbf{w}$ using a truncated (simplex) multivariate normal distribution as

$$\mathbf{w}_l \sim N_{[0,1]^{K-1}}\left(\mathbf{w}, \psi^2 \cdot I\right)$$

The mixture weight covariance is an identity matrix scaled by a mixture variance ψ^2.

The template mixture weight $\mathbf{w}$ is assigned a uniform prior probability over the K contributor simplex.

$$\mathbf{w} \sim Dir(\mathbf{1})$$

The mixture variance ψ^2 has an inverse gamma prior probability distribution.

$$\psi^{-2} \sim Gam(1/2, 1/200)$$

Genotype Combination

Genotypes are combined in a mixture by adding together contributor vectors, with each contributor weighted by its mixture weight. The sum is a genotype vector that describes the total number of alleles in the sample at each fragment size.

A quantitative linear model of data pattern $\mathbf{d}_l$ at locus l has an expected vector value μ_l given by the weighted genotype sum

$$\mu_l = m_l \cdot \sum_{k=1}^{K} w_{k,l} \cdot \mathbf{g}_{k,l}$$

Amplification Variance

The polymerase chain reaction (PCR) is an imperfect copying mechanism. A PCR cycle does not automatically double the number of copies of a particular DNA fragment. Rather, the number of fragment copies randomly increases each round by a factor between one and two. This random branching process follows the mathematics of a Poisson counting process, which can be modeled as a positive-valued distribution having a variance that scales with fragment quantity y as $\sigma^2 \cdot y$.

The data variation parameter σ^2 has an inverse gamma prior probability distribution.

$$\sigma^{-2} \sim Gam(10, 20)$$

Background Variance

Instrument noise arises from a genetic analyzer's laser signal, optical path, CCD camera, and other sources. This background noise is independent of the PCR process, and can be modeled as a normal distribution having a fixed variance parameter.

The data variation parameter τ^2 has an inverse gamma prior probability distribution.

$$\tau^{-2} \sim Gam(10, 500)$$

PCR Stutter

The DNA polymerase enzyme can drop or add a repeated STR unit when replicating an STR fragment. The Markov chain process forms a random pattern of fragment lengths centered about the primary allele length. This PCR *stutter* pattern is far more pronounced with the mono- or di-nucleotide repeat loci used in genetics, and attenuated somewhat with the tetra- or penta-nucleotide repeats used in forensics.

The stutter amount increases with the number of repeats, and can be modeled as a regression line. Let x be the number of repeat units, and y the stutter proportion. Then the linear model relating increasing stutter amount to repeat length at a locus is:

$$y \sim N\left(a + bx, \sigma_s^2\right)$$

Prior probabilities for the PCR stutter model parameters are:

$$a \sim N(0,1)$$
$$b \sim N\left(0, 10^{-6}\right)$$
$$\sigma_s^{-2} \sim Gam\left(0.5, 0.5 \cdot 10^{-2}\right)$$

The stutter proportion is constrained to lie between 0% and 15%.

Relative Amplification

PCR amplifies shorter DNA fragments more efficiently than longer ones. This *relative amplification* displaces allele mass away from longer alleles toward short ones.

The allele mass rebalancing increases with the size difference between alleles, and can be modeled as normally distributed variation in allele height. Let Δx be the difference in repeat units, and Δy the difference in allele peak heights. Then the linear model relating allele height difference to size difference at a locus is:

$$\Delta y \sim N\left(c \cdot \Delta x, \sigma_R^2\right)$$

Prior probabilities for the relative amplification model parameters are:

$$c \sim N\left(0, 10^{-4}\right)$$
$$\sigma_R^{-2} \sim Gam\left(0.5, 0.5 \cdot 10^{-6}\right)$$

Differential Degradation

Polymerase requires a connected DNA fragment in order to make a copy. One or more breaks in a DNA sequence will prevent PCR copying. The chance of having no breaks in a fragment (unimpeded copying) follows an exponential decay curve in the fragment length variable, with a decay rate proportional to the density of DNA breaks.

Since TrueAllele models the DNA mass and variation of each experiment separately, no additional modeling is needed when DNA degradation or inhibition is the same for all contributors. However, when there is differential degradation between the different contributors, the decay rate of each contributor's DNA can be determined by logarithmic modeling of the exponential process.

Let x be allele size, y contributor allele amount, and y_{eff} the effective contributor amount following DNA degradation. Then the linear model relating effective allele amount to allele size for a contributor at a locus is:

$$\log\left(\frac{y_{eff}}{y}\right) \sim N\left(-\lambda \cdot x, \sigma_D^2\right)$$

Prior probabilities for the differential degradation model parameters are:

$$\lambda \sim N_+\left(0, 10^{-6}\right)$$
$$\sigma_D^{-2} \sim Gam\left(0.5, 0.5 \cdot 10^{-2}\right)$$

Hierarchical Modeling

TrueAllele models variables hierarchically, subdividing them by experiment. Thus one parameter can expand into many parameters, one for each STR locus experiment, and another one for the group. This expansion of variables permits modeling that is more customized to the data, yielding more accurate answers.

For example, contributor mixture weights are determined for each locus experiment as the set of variables $\{\mathbf{w}_l\}$, and also for the DNA template as group variable $\mathbf{w}$.

$$\mathbf{d}_l \sim N_+\left(m_l \cdot \sum_{k=1}^{K} w_{k,l} \cdot \mathbf{g}_{k,l}, \Sigma_l\right)$$

$$\mathbf{w}_l \sim N_{[0,1]^{K-1}}\left(\mathbf{w}, \psi^2 \cdot I\right)$$

$$\mathbf{w} \sim Dir(\mathbf{1})$$

Statistical Computing

The joint probability distribution is fully specified as the product of the likelihood and prior distributions. Using a Metropolis-Hastings sampler, we iteratively draw from the posterior probability distributions of $\{\mathbf{g}_{k,l}\}$, $\{\mathbf{w}_l\}$, $\{m_l\}$, $\mathbf{w}$, σ^2, τ^2, ψ^2 and other variables using Markov chain Monte Carlo (MCMC) computer methods.

Once beyond the initial burn in phase, the Markov chain samples from the joint posterior probability distribution. Marginalizing these posterior samples to each genotype

random variable $\mathbf{g}_{k,l}$ for contributor k at locus l, we obtain the desired posterior probability functions $q(x)$ for the genotypes.

Match Statistic

The likelihood ratio (LR) is the information gained in the hypothesis H odds by having observed data

$$LR = \frac{O\left(H \middle| d_Q, d_R, d_S\right)}{O(H)}$$

Here, hypothesis H is that the suspect contributed to the DNA evidence, and the DNA data comprises the questioned evidence d_Q, the reference population allele frequencies d_R and suspect profile d_S.

Standard Bayesian rearrangements tell us that the LR can also be written as the ratio of conditional probabilities

$$LR = \frac{\Pr\left\{d_Q \middle| H, d_R, d_S\right\}}{\Pr\left\{d_Q \middle| \overline{H}, d_R, d_S\right\}}$$

where $\overline{H}$ is the alternative hypothesis that someone else contributed to the evidence.

Suppose that there is uncertainty in the evidence genotype having pmf $q(x)$ or in the suspect genotype with pmf $s(x)$. Then this genotype uncertainty is expressed in the LR as

$$LR = \frac{\sum_{x \in G} \lambda_Q(x) \cdot s(x)}{\sum_{x \in G} \lambda_Q(x) \cdot r(x)}$$

where $\lambda_Q(x)$ is the likelihood function of the evidence genotype Q and $r(x)$ is the pmf of reference population genotype.

Bayes theorem lets us rewrite this ratio of likelihood sums as a numerically equivalent sum of posterior genotype probability product ratios. Probability can be more intuitive and easier to explain than likelihood.

$$LR = \sum_{x \in G} \frac{q(x) \cdot s(x)}{r(x)}$$

This genotype probability formulation expresses the LR as a sum of ratios that compare match probability to coincidence.

Co-ancestry Correction

The LR for the hypothesis that a person contributed their DNA to evidence items 1 and 2 is calculated from genotype probability distributions via:

$$LR = \frac{\sum_{x \in G} \lambda_1(x) \cdot \lambda_2(x) \cdot \pi_\theta(x)}{\sum_{x \in G} \sum_{y \in G} \lambda_1(x) \cdot \lambda_2(y) \cdot \pi_\theta(x,y)}$$

The joint prior probability $\pi_\theta(x,y)$ function is just the product of independent population priors $\pi(x)$ and $\pi(y)$ when not accounting for co-ancestry (i.e., $\theta = 0$). However, it is more accurate and conservative to recognize that people in a human population share common ancestors (i.e., $\theta > 0$).

The conditional match formulae for the homozygote and heterozygote cases developed by Balding and Nichols were given in the National Research Council (NRC) II report equations 4.10a and 4.10b, derivable from the probability ratio $\pi_\theta(x,y)/\pi_\theta(x)$. The corresponding joint prior probabilities $\pi_\theta(x,y)$ at a particular value of θ are:

$$\pi_\theta(aa,aa) = \frac{p_a\left[(1-\theta)p_a + \theta\right]\left[(1-\theta)p_a + 2\theta\right]\left[(1-\theta)p_a + 3\theta\right]}{(1+\theta)(1+2\theta)}$$

$$\pi_\theta(ab,ab) = \frac{4p_a\left[(1-\theta)p_a + \theta\right]p_b\left[(1-\theta)p_b + \theta\right](1-\theta)}{(1+\theta)(1+2\theta)}$$

In situations where the genotype allele pair values are not the same, the joint probabilities $\pi_\theta(x,y)$ can be similarly calculated from their Dirichlet distributions, as described in Chapter 4 of Evett and Weir's DNA interpretation textbook.

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EXHIBIT 13

COMMONWEALTH of VIRGINIA

DEPARTMENT OF FORENSIC SCIENCE

OFFICE OF THE DIRECTOR
DEPARTMENT OF FORENSIC SCIENCE
A Nationally Accredited Laboratory

700 NORTH 5TH STREET
RICHMOND, VIRGINIA 23219
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April 4, 2016

Daniel T. Satterberg, Prosecuting Attorney
W554 King County Courthouse
516 Third Avenue
Seattle, Washington 98104

Re: The Virginia Department of Forensic Science Validation of TrueAllele® Casework

Dear Mr. Satterberg,

I am employed at the Virginia Department of Forensic Science (DFS) as a Forensic Molecular Biologist. TrueAllele® Casework was validated by our laboratory prior to its use on forensic casework. As part of my job responsibilities with DFS, I was the principal scientist on the validation study for TrueAllele®.

The validation of any new technology by a forensic laboratory is necessary and required prior to its implementation and use for analysis of evidence. The primary aims of validation work are to determine if the product performs as advertised, to develop an expertise in the use of the product, to assess the accuracy, precision and reproducibility (where applicable) of the technology, and to understand its limitations.

We have achieved these goals without the source code to TrueAllele® Casework, as is true for the many different technologies and products that we use daily in the laboratory. Testing TrueAllele® Casework using complex DNA profiles where we knew the answer (i.e., the genetic makeup of the contributors to the DNA profiles) provided invaluable information. Moreover, simple statistical calculations performed by TrueAllele® Casework were compared to values produced by another previously validated software program and the comparison aided our laboratory in the validation process. This internal validation work informed us as to the accuracy and reproducibility of the process, the limitations of the system, the ability of the technology to detect minor contributors to the DNA profiles (sensitivity) and perhaps most importantly, the ability to eliminate non-contributors to the DNA profiles (specificity).

Our ability to use a given technology for forensic DNA profiling is verified by thorough validation work, not studying the source code. We have never requested the source

code for the TrueAllele® Casework software because it was not necessary in order to determine the reliability of TrueAllele® Casework.

I have attached a copy of our published validation study for your reference.

Sincerely,

Susan A. Greenspoon, Ph.D.
Forensic Molecular Biologist

EXHIBIT 14

frontiers in Genetics

GENERAL COMMENTARY
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Commentary: A "Source" of Error: Computer Code, Criminal Defendants, and the Constitution

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A commentary on

A "Source" of Error: Computer Code, Criminal Defendants, and the Constitution
by Chessman, C. (2017). Calif. Law Rev. 105, 101–193.

Chessman (2017) warns of the current trend to admit into court unchallenged the results of complex computerized calculations. He provides a number of examples and arguments claimed to demonstrate the need for open source software to remove the "black box" element. We agree with parts of this sentiment, and the topic of this special issue, that there is a danger with those using and receiving information from black box systems.

Some care however is needed with simple diagnoses and prescriptions such as these.

Modern probabilistic genotyping software are replacing methods previously applied manually. We have great confidence in the forensic community with regard to both integrity and dedication. The previously applied processes are usually a composite of standard operating procedure and human judgment. The difference between these and probabilistic software is largely that the processes in the software are encoded.

Many disciplines are sufficiently broad that practitioners need to rely, in part, on the work of others. This is not new (for a discussion on this point see Taylor, 2016). The risk to which Chessman refers arises when the individual using the system has so little understanding that they do not know how to use the system, or when it has not worked[1]. Chessman provides some helpful suggestions for how breaking down black box barriers can be addressed on an individual and systemic scale. As developers of expert system STRmix™[2] (Taylor et al., 2013), we wish to address some of the alarmist points in Chessman (and echoed by others[3]) that gives the impression that producers of expert systems are all either incompetent or corrupt.

We first wish to correct a couple of points in (Chessman, 2017). Regarding the "erroneous assumption" referenced by footnotes 49–51: This miscode, and indeed any miscode found that has been identified in STRmix™ development or use, was identified by examination of the program's output and not the source code. It would be nearly impossible to identify subtle errors in code by viewing the code. The identification has always been a result of comparison of the results produced by a program to some known control[4]. The results of these comparisons then trigger the examination of a specific section of the code in order to discover the source of the discrepancy.

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[1] Note that this is not an issue with just computer programs, recent history has numerous examples within forensic biology showing that a misunderstanding of the way a system works at a fundamental level can cause issues even when the calculations themselves are relatively simple and able to be done by hand (Budowle and Bieber, 2015).

[2] An expert system that analyses STR DNA profile data.

[3] For example see EPIC (https://epic.org/state-policy/foia/dna-software/).

[4] Commonly a "by-hand" recreation of the expected value(s).

Even as developers, during the developmental validation of new versions of STRmix™, we utilize the extended outputs of the software to validate, and do not validate by examination of code. A further reference (footnote 98) makes the same incorrect assumption that it was code review that lead to the discovery of a programming error. Our experience has been that even more crucial than a review of source code, is the ability to have access to outputs that demonstrate each step of a calculation. We should also note that our ongoing evaluation and testing of the software is a marker of continuous validation and refinement, rather than just fixing "errors" and "blunders."

The second point we wish to make is that the type and magnitude of miscodes are important to consider. The majority of programming errors will lead to instances of a program "crashing" or failing to produce an answer. These types of errors are arguably inconsequential as they will not lead to any erroneous results being produced. More serious are miscodes where no errors are identified or displayed by the software. These can be split into those that will be clearly identifiable[5] and those that are more subtle and may go initially unnoticed. Even in this latter category, the question should be asked "What effect does this error have?" If the magnitude of the difference in the result caused by the miscode is small compared with the natural variability in the results being produced[6] then arguably the consequences are minimal. We are by no means suggesting that that these types of errors are acceptable, they should be rectified as soon as found. We simply suggest that they tend to be used for scaremongering in a manner disproportionate to their impact. Case in point is the oft quoted article (David Murray, 2015), which contains the never quoted sentence "*The DNA likelihood ratios in both the new and original statements appear to be the same.*"

We agree with the suggestion of Chessman that source code should be available for scrutiny. STRmix™ abides by one of the mechanisms that Chessman suggests, namely the ability for code to be disclosed under confidentiality agreements[7]. We note that running of STRmix™ is just the final step in a long journey of computerized activities that ultimately lead to an answer. A true challenge of all steps in the process would require the examination of the source code underlying the Java programming language in which STRmix™ is written, the Windows™ operating system on which it is run, the software used to process the raw electrophoretic data, the software used to collect the raw electrophoretic data from the electrophoresis instrument, the code used to run the electrophoresis instrument, the PCR thermocycler, the quantification instrument and a myriad of no doubt thousands of blocks of code that sit within the numerous Peripheral Interface Controllers that control hardware components.

With the advent of complex computerized evaluation of evidence, there is a shift from a time where an expert can testify to all aspects of the evaluation, to one where, at some level, the workings of an expert system are accepted without absolute understanding. This may initially seem frightening, but an examination of the bigger picture suggests otherwise. It would be difficult to argue that the use of computerized breathalyzers is a backwards step from the reliability of the Field Sobriety Test. Similarly, virtually all senior advisory bodies relating to DNA profile evaluation recognize the clear benefits of the probabilistic interpretation systems (which by nature of their complexity require computerized implementation) over the preceding manual or binary interpretation methods (Coble et al., 2015; SWGDAM, 2015). In our efforts to ensure that software is not the "source" of errors, it is important to recognize that even with the noted occurrences of these errors, the current computerized solutions, when used by trained experts, represent a vast improvement to the quality and reliability of evidence presented in court.

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All authors contributed to the discussions and writing of the manuscript. Points of view in this document are those of the authors and do not necessarily represent the official position or policies of the author's organizations.

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[5]Such as value of a probability greater than one, or a negative amount of some substance.

[6]This may either be in the raw results due to inherent variability in the laboratory process or it may be variability in the statistical result due to an evaluation method that utilizes random number generation (Bright et al., 2015).

[7]The code of STRmix™ has been viewed under such conditions in the past.

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EXHIBIT 15

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IN THE COURT OF APPEAL OF THE STATE OF CALIFORNIA

SECOND APPELLATE DISTRICT

DIVISION FOUR

THE PEOPLE, Petitioner, v. THE SUPERIOR COURT OF LOS ANGELES COUNTY, Respondent; MARTELL CHUBBS, Real Party in Interest.	B258569 (Los Angeles County Super. Ct. No. NA093179)

ORIGINAL PROCEEDINGS in mandate. Richard R. Romero, Judge. Writ granted.

Jackie Lacey, District Attorney, Roberta Schwartz and Matthew Brown, Deputy District Attorneys, for Petitioner.

No appearance for Respondent.

Angelyn Gates for Real Party in Interest.

Real party in interest Martell Chubbs was charged in a November 28, 2012 information with the murder of Shelley H. in 1977 (Pen. Code, § 187, subd. (a)).[1] The charge was filed after a DNA sample from the victim was found to be a match for Chubbs. The People petition for a writ of mandate to overturn the order of the superior court compelling the disclosure of a computer source code for software, TrueAllele® Casework (TrueAllele), which was used in the DNA analysis. The People contend that the source code is a protected trade secret of the creator and owner of the software, Mark W. Perlin, and his company, Cybergenetics. We grant the petition.

FACTUAL AND PROCEDURAL BACKGROUND

Preliminary Hearing Evidence[2]

In December 1977, Long Beach Police Department officers found the 17-year-old victim in her Long Beach apartment. She was lying on the end of the bed with her feet touching the ground and with an electrical wire tied around her neck. During an autopsy, swabs were taken from the victim's vagina and smeared onto slides.

In June 2011, as part of a cold case investigation, Sorenson Forensics (Sorenson) conducted a DNA test on the vaginal swabs from the victim. Sorenson generated a DNA report that indicated three contributors to the DNA: a major sperm DNA profile attributable to an unidentified male, a minor sperm DNA

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1 All unspecified statutory references are to the Penal Code.

2 The People have not included the transcript of the preliminary hearing, instead relying on a declaration from the deputy district attorney who appeared at the preliminary hearing, summarizing the evidence.

profile, and a partial DNA profile attributable to the victim. Sorenson excluded the victim's husband, Nolan Hankins, as the source of the major sperm DNA profile.[3]

Although the record before us does not include the basis for the arrest, Long Beach Police Department detectives arrested Chubbs in August 2012. Chubbs confirmed that he lived in Long Beach in the 1970s.

In September 2012, Sorenson compared the DNA profile of Chubbs, an African-American, to the major sperm DNA profile and found a match. The frequency of the profile occurrence in the general population was determined to be one in approximately 10,000 for African Americans.

At the preliminary hearing in November 2012, Chubbs was held to answer for one count of murder. The information charged Chubbs with one count of murder and alleged six prior convictions of serious felonies (§ 667, subd. (a)(1)) that also qualified as strikes under the Three Strikes law (§§ 667, subds. (b)-(i), 1170.12, subds. (a)-(d)). In January 2013, Chubbs pleaded not guilty to the murder charge.

As part of trial preparation, in September 2013, the People sent the victim's vaginal slide to Cybergenetics' lab in Pittsburgh, Pennsylvania for further testing. Cybergenetics prepared a supplemental report, explaining that it had used its TrueAllele software to "infer possible DNA contributor genotypes from the samples," then compared the evidence genotypes to the reference genotypes (which included Chubbs' and Hankins' genotypes) to compute likelihood ratio DNA match statistics. "TrueAllele assumed that the evidence sample data . . . contained two or three contributors, and objectively inferred evidence genotypes solely from these data." Perlin concluded in the supplemental report that the DNA

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[3] Hankins is sometimes referred to in the record as the victim's boyfriend, rather than her husband.

match between the vaginal sperm sample and Chubbs is "1.62 quintillion times more probable than a coincidental match to an unrelated Black person." Perlin also concluded that the DNA match with Hankins was "2.82 million times more probable than a coincidental match to an unrelated Black person."

Defense Discovery Efforts

In November 2013, Chubbs made his third informal discovery request, which included the request at issue here, for Cybergenetics' source codes for TrueAllele. In January 2014, Chubbs filed a motion to compel discovery that included the request for Cybergenetics' source codes. Defense counsel cited statements in Cybergenetics' supplemental report indicating that TrueAllele made assumptions and inferences in computing its DNA match statistics. According to defense counsel, the TrueAllele program was "brand new" and had not been the subject of a *Kelly* hearing, and without the source codes there would be no way to cross examine Perlin about the efficacy and accuracy of the program.[4]

The defense received several discovery items related to Cybergenetics and TrueAllele, including the following: the September 2013 supplemental report, a November 2013 case packet by Cybergenetics, published articles by Perlin

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[4] The three-pronged test established in *People v. Kelly* (1976) 17 Cal.3d 24 "provides a framework within which courts can analyze the reliability of expert testimony based on new or novel scientific methods or techniques." (*People v. Lucas* (2014) 60 Cal.4th 153, 223.) "'The first prong requires proof that the technique is generally accepted as reliable in the relevant scientific community. [Citation.] The second prong requires proof that the witness testifying about the technique and its application is a properly qualified expert on the subject. [Citation.] The third prong requires proof that the person performing the test in the particular case used correct scientific procedures.' [Citation.]" (*Id.* at p. 223, fn. 31.) The test is also known as the *Kelly/Frye* test. (*In re Jordan R.* (2012) 205 Cal.App.4th 111, 115, fn. 3; see *Frye v. U.S.* (D.C. Cir. 1923) 293 F. 1013.)

regarding DNA analysis and the TrueAllele software, a data disc from Sorenson, TrueAllele manuals from March 2014, a data disc from Cybergenetics, and a PowerPoint presentation to be used by Perlin. However, the dispute here focuses on the source codes for TrueAllele, which were not produced.

On January 15, 2014, the People filed an opposition to the motion to compel discovery, arguing that the defense was not entitled to a discovery order because the People had voluntarily complied with their discovery obligations, citing section 1054.5, subdivision (a).[5] As pertinent here, the People explained that they requested the source code from Cybergenetics, but Cybergenetics did not turn it over because it is a trade secret. The People argued that disclosure of the source code would be "financially devastating" to Cybergenetics.

The People stated in their opposition that, although Cybergenetics is unwilling to disclose its source code, it "is willing to conduct additional TrueAllele testing on a limited set of defense-provided data to further defense understanding of the system, its operation and its reliability. Cybergenetics is also willing to meet with defense experts (in person or via an Internet meeting) to show them the results in this case, and explain to them on a TrueAllele computer how the system operates, though Cybergenetics cannot provide [the] defense with a[n] executable version of the TrueAllele casework system which costs $60,000."

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[5] The statute provides: "No order requiring discovery shall be made in criminal cases except as provided in this chapter. This chapter shall be the only means by which the defendant may compel the disclosure or production of information from prosecuting attorneys, law enforcement agencies which investigated or prepared the case against the defendant, or any other persons or agencies which the prosecuting attorney or investigating agency may have employed to assist them in performing their duties." (§ 1054.5, subd. (a).)

Chubbs then filed an application for an out-of-state subpoena duces tecum, seeking the source codes for the TrueAllele software. He argued that the source codes were essential to his defense because the DNA evidence from the vaginal slide was the only evidence against him. He pointed out the discrepancy between the random match probability calculated by Sorenson (1 in approximately 10,000) and the likelihood ratio calculated by Cybergenetics (1.62 quintillion times more probable than a coincidental match) to argue that the source codes were necessary to cross-examine Perlin about the accuracy of TrueAllele.

In a declaration submitted with the application, defense counsel stated that forensic experts and other attorneys who work with DNA evidence advised her to obtain the source codes for TrueAllele. She stated that "other experts in the field have developed a similar software program as TrueAllele for which their source codes are open for public review." Defense counsel further stated that Allan Jamieson, an expert in DNA analysis who had experience with TrueAllele, told her that she could not properly defend against the TrueAllele results without the source codes.

Jamieson stated in his declaration that "access to this code is the only satisfactory and professionally recommended way to fully consider the validity of the TrueAllele analysis" in this case. He stated that "[o]ther analysts who have developed computer-assisted DNA comparison software . . . do not hide their source codes" and instead make them freely available, which allows others to fully review and verify the reliability of the method and results in any given case.

Motion to Quash

On May 16, 2014, the People filed a motion to quash the subpoena duces tecum. Contrary to its earlier argument in its opposition to the motion to compel

discovery, the People now argued that Cybergenetics was not a third party to the investigation but instead was an investigatory agency within the meaning of section 1054.5, subdivision (a).

The trial court denied the People's motion to quash and issued a certificate for an out-of-state subpoena, ordering Perlin to produce the source codes. On June 16, 2014, a Pennsylvania court issued an order directing compliance with the subpoena duces tecum. The Pennsylvania court reasoned that Perlin was a material witness, and the means by which he arrived at his opinions likewise was material. The court thus ordered Perlin to appear with the source codes and stated that any issue regarding the disclosure of trade secrets should be determined by the California court.

The People moved to quash the subpoena duces tecum. The People argued that the materials are a trade secret, that Chubbs has not established the source codes are material or necessary, and that the discovery is not permitted by section 1054, subdivision (e).[6]

On June 24, 2014, the trial court issued and held a body attachment for Perlin based on his failure to appear pursuant to the subpoena duces tecum. The People subsequently withdrew the contention that Perlin was an expert employed by the prosecution pursuant to subdivision (a) of section 1054.5, noting that Perlin had retained private counsel regarding the trade secret privilege.

Perlin, represented by California counsel, submitted a brief in support of his assertion of the trade secret privilege. The People filed a motion to reconsider the

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[6] Section 1054 states that the chapter on discovery "shall be interpreted to give effect to all of the following purposes," including "[t]o provide that no discovery shall occur in criminal cases except as provided by this chapter, other express statutory provisions, or as mandated by the Constitution of the United States." (§ 1054, subd. (e).)

court's May 16 order denying the People's motion to quash the subpoena duces tecum, and for an order granting the motion and quashing the body attachment held for Perlin.

At a July 29, 2014 hearing, the court ruled the source codes are not necessary pursuant to *Kelly/Frye,* but that Chubbs' right to confront and cross-examine witnesses required the production of the source codes. The prosecutor again invoked the trade secret privilege on Perlin's behalf. The court found that nondisclosure of the source codes does "work injustice" in the sense that it denies Chubbs a right to confront and cross-examine witnesses (Evid. Code, § 1060), and that a protective order can protect Perlin's interest. The court indicated that it would follow the procedure set forth in Evidence Code sections 1061 and 1062, by issuing a protective order and, if needed, excluding the public from the proceedings. The court held the body attachment for Perlin until August 26 and ordered the prosecution to provide the source codes on that date.

On August 26, 2014, the court deemed the TrueAllele source code a trade secret for purposes of the trial. Perlin brought an encrypted form of the source code. However, before turning over the source code, the prosecution raised the issue of a protective order. The court explained that although it would grant a protective order to minimize disclosure of the source code, the source code would be revealed to a certain extent at trial. The People subsequently did not proffer a protective order, but instead refused to turn over the source code. Defense counsel requested the exclusion of the TrueAllele results at trial. The court granted the request based on Chubbs' rights under the confrontation clause and the fact Perlin was to be a main prosecution witness against Chubb.

The People petitioned for a writ of mandate to this court. We issued an alternative writ of mandate ordering the superior court to vacate the July 29 and

August 26, 2014 orders compelling the disclosure of the computer source codes, or to show cause why a peremptory writ of mandate should not issue.[7] The superior court did not vacate its ruling, and the matter is now before us.

DISCUSSION

The People contend that the trial court improperly applied the trade secret privilege and that Chubbs failed to make a prima facie showing sufficient to overcome the privilege.[8] "The court's ruling on a discovery motion is subject to review for abuse of discretion. [Citation.]" (*People v. Jenkins* (2000) 22 Cal.4th 900, 953.) "A trial court has abused its discretion in determining the applicability of a privilege when it utilizes the wrong legal standards to resolve the particular issue presented. [Citation.]" (*Seahaus La Jolla Owners Assn. v. Superior Court* (2014) 224 Cal.App.4th 754, 766.)

We begin by setting forth the statutes and law regarding the trade secret privilege.

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7 We further issued a temporary stay of the trial.

8 We disagree with Chubbs' contention that the trial court's ruling was an evidentiary ruling not subject to writ review. Although the trial court's ultimate ruling was to exclude the TrueAllele evidence, this was based on the People's refusal to disclose the source codes. "'Extraordinary review of a discovery order will be granted when a ruling threatens immediate harm, such as loss of a privilege against disclosure, for which there is no other adequate remedy. [Citation.] . . . "'[W]here the petitioner seeks relief from a discovery order that may undermine a privilege, we review the trial court's order by way of extraordinary writ. [Citation.]'" [Citation.]' [Citation.]" (*Doe v. Superior Court* (2011) 194 Cal.App.4th 750, 754; see also § 1512, subd. (a) [authorizing the people to seek review of an order granting a defendant's motion for discovery by a petition for a writ of mandate]; *People v. Superior Court* (*Mouchaourab*) (2000) 78 Cal.App.4th 403, 413 ["writ review is appropriate when the petitioner 'seeks relief from a discovery order which may undermine a privilege, because appellate remedies are not adequate once the privileged information has been disclosed'"].)

I. *Trade Secret Privilege*

Evidence Code section 1060 provides: "If he or his agent or employee claims the privilege, the owner of a trade secret has a privilege to refuse to disclose the secret, and to prevent another from disclosing it, if the allowance of the privilege will not tend to conceal fraud or otherwise work injustice." In the instant case, it is undisputed that the source codes in issue constitute a trade secret. (See Evid. Code, § 1062, subd. (a) [for purposes of Evidence Code sections 1061, 1062, and 1063, which apply to criminal cases, "[t]rade secret" is defined in Civil Code section 3426.1 or Penal Code section 499c, subdivision (a)(9)].)

In civil cases, a burden-shifting procedure is used to evaluate assertion of the trade secret privilege. Based on the language and legislative history of Evidence Code section 1060, the court in *Bridgestone/Firestone, Inc. v. Superior Court* (1992) 7 Cal.App.4th 1384 (*Bridgestone*) held that "the party claiming the [trade secret] privilege has the burden of establishing its existence. [Citations.] Thereafter, the party seeking discovery must make a prima facie, particularized showing that the information sought is relevant and necessary to the proof of, or defense against, a material element of one or more causes of action presented in the case, and that it is reasonable to conclude that the information sought is essential to a fair resolution of the lawsuit. It is then up to the holder of the privilege to demonstrate any claimed disadvantages of a protective order." (*Id.* at p. 1393; see also *Raymond Handling Concepts Corp. v. Superior Court* (1995) 39 Cal.App.4th 584, 590-591 [relying on the procedure enunciated in *Bridgestone* and concluding that the information was discoverable and that the trial court did not abuse its discretion in entering a protective order].)

Chubbs contends that when a defendant in a criminal case seeks disclosure of an item meeting the definition of a trade secret, Evidence Code section 1060 does not permit the owner of the trade secret to refuse to disclose. Rather, according to Chubbs, Evidence Code sections 1061 and 1062 supersede section 1060, and authorize only (on a proper showing) the remedy of a protective order (§ 1062) and exclusion of the public from portions of the trial at which a trade secret might be revealed (§ 1062). Evidence Code section 1061 provides: "In addition to Section 1062, the following procedure shall apply whenever the owner of a trade secret wishes to assert his or her trade secret privilege, as provided in Section 1060, during a criminal proceeding." (Evid. Code, § 1061, subd. (b).) The statute then sets forth a procedure under which the holder of the trade secret privilege or an authorized representative may move for a protective order (Evid. Code, § 1061, subd. (b)(1)), any party to the proceeding may oppose the motion (*id.*, subd. (b)(2)), and the court, on a finding "that a trade secret may be disclosed . . . unless a protective order is issued and that the issuance of a protective order would not conceal a fraud or work an injustice, . . . issue[s] a protective order limiting the use and dissemination of the trade secret" (*id.*, subd. (b)(4)).[9]

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[9] Evidence Code section 1061 provides in relevant part: "(b) In addition to Section 1062, the following procedure shall apply whenever the owner of a trade secret wishes to assert his or her trade secret privilege, as provided in Section 1060, during a criminal proceeding:

"(1) The owner of the trade secret shall file a motion for a protective order, or the people may file the motion on the owner's behalf and with the owner's permission. The motion shall include an affidavit based upon personal knowledge listing the affiant's qualifications to give an opinion concerning the trade secret at issue, identifying, without revealing, the alleged trade secret and articles which disclose the secret, and presenting evidence that the secret qualifies as a trade secret under either subdivision (d) of Section 3426.1 of the Civil Code or paragraph (9) of subdivision (a) of Section 499c of the Penal Code. The motion and affidavit shall be served on all parties in the proceeding.

"(2) Any party in the proceeding may oppose the request for the protective order by submitting affidavits based upon the affiant's personal knowledge. The affidavits shall be filed under seal, but shall be provided to the owner of the trade secret and to all parties in the proceeding. Neither the owner of the trade secret nor any party in the proceeding may disclose the affidavit to persons other than to counsel of record without prior court approval.

"(3) The movant shall, by a preponderance of the evidence, show that the issuance of a protective order is proper. The court may rule on the request without holding an evidentiary hearing. However, in its discretion, the court may choose to hold an in camera evidentiary hearing concerning disputed articles with only the owner of the trade secret, the people's representative, the defendant, and defendant's counsel present. If the court holds such a hearing, the parties' right to examine witnesses shall not be used to obtain discovery, but shall be directed solely toward the question of whether the alleged trade secret qualifies for protection.

"(4) If the court finds that a trade secret may be disclosed during any criminal proceeding unless a protective order is issued and that the issuance of a protective order would not conceal a fraud or work an injustice, the court shall issue a protective order limiting the use and dissemination of the trade secret, including, but not limited to, articles disclosing that secret. The protective order may, in the court's discretion, include the following provisions:

"(A) That the trade secret may be disseminated only to counsel for the parties, including their associate attorneys, paralegals, and investigators, and to law enforcement officials or clerical officials.

"(B) That the defendant may view the secret only in the presence of his or her counsel, or if not in the presence of his or her counsel, at counsel's offices.

"(C) That any party seeking to show the trade secret, or articles containing the trade secret, to any person not designated by the protective order shall first obtain court approval to do so:

"(i) The court may require that the person receiving the trade secret do so only in the presence of counsel for the party requesting approval.

"(ii) The court may require the person receiving the trade secret to sign a copy of the protective order and to agree to be bound by its terms. The order may include a

Evidence Code section 1062 similarly provides a procedure under which "the court, upon motion of the owner of a trade secret, or upon motion by the People with the consent of the owner, may exclude the public from any portion of a criminal proceeding where the proponent of closure has demonstrated a substantial probability that the trade secret would otherwise be disclosed to the public during that proceeding and a substantial probability that the disclosure would cause serious harm to the owner of the secret, and where the court finds that there is no

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provision recognizing the owner of the trade secret to be a third-party beneficiary of that agreement.

"(iii) The court may require a party seeking disclosure to an expert to provide that expert's name, employment history, and any other relevant information to the court for examination. The court shall accept that information under seal, and the information shall not be disclosed by any court except upon termination of the action and upon a showing of good cause to believe the secret has been disseminated by a court-approved expert. The court shall evaluate the expert and determine whether the expert poses a discernible risk of disclosure. The court shall withhold approval if the expert's economic interests place the expert in a competitive position with the victim, unless no other experts are available. The court may interview the expert in camera in aid of its ruling. If the court rejects the expert, it shall state its reasons for doing so on the record and a transcript of those reasons shall be prepared and sealed.

"(D) That no articles disclosing the trade secret shall be filed or otherwise made a part of the court record available to the public without approval of the court and prior notice to the owner of the secret. The owner of the secret may give either party permission to accept the notice on the owner's behalf.

"(E) Other orders as the court deems necessary to protect the integrity of the trade secret.

"(c) A ruling granting or denying a motion for a protective order filed pursuant to subdivision (b) shall not be construed as a determination that the alleged trade secret is or is not a trade secret as defined by subdivision (d) of Section 3426.1 of the Civil Code or paragraph (9) of subdivision (a) of Section 499c of the Penal Code. Such a ruling shall not have any effect on any civil litigation."

overriding public interest in an open proceeding. No evidence, however, shall be excluded during a criminal proceeding pursuant to this section if it would conceal a fraud, work an injustice, or deprive the People or the defendant of a fair trial." (Evid. Code, § 1062, subd. (a).)[10]

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[10] Section 1062 provides in full: "(a) Notwithstanding any other provision of law, in a criminal case, the court, upon motion of the owner of a trade secret, or upon motion by the People with the consent of the owner, may exclude the public from any portion of a criminal proceeding where the proponent of closure has demonstrated a substantial probability that the trade secret would otherwise be disclosed to the public during that proceeding and a substantial probability that the disclosure would cause serious harm to the owner of the secret, and where the court finds that there is no overriding public interest in an open proceeding. No evidence, however, shall be excluded during a criminal proceeding pursuant to this section if it would conceal a fraud, work an injustice, or deprive the People or the defendant of a fair trial.

"(b) The motion made pursuant to subdivision (a) shall identify, without revealing, the trade secrets which would otherwise be disclosed to the public. A showing made pursuant to subdivision (a) shall be made during an in camera hearing with only the owner of the trade secret, the People's representative, the defendant, and defendant's counsel present. A court reporter shall be present during the hearing. Any transcription of the proceedings at the in camera hearing, as well as any articles presented at that hearing, shall be ordered sealed by the court and only a court may allow access to its contents upon a showing of good cause. The court, in ruling upon the motion made pursuant to subdivision (a), may consider testimony presented or affidavits filed in any proceeding held in that action.

"(c) If, after the in camera hearing described in subdivision (b), the court determines that exclusion of trade secret information from the public is appropriate, the court shall close only that portion of the criminal proceeding necessary to prevent disclosure of the trade secret. Before granting the motion, however, the court shall find and state for the record that the moving party has met its burden pursuant to subdivision (b), and that the closure of that portion of the proceeding will not deprive the People or the defendant of a fair trial.

"(d) The owner of the trade secret, the People, or the defendant may seek relief from a ruling denying or granting closure by petitioning a higher court for extraordinary relief.

Although Chubbs does not expressly acknowledge it, an implicit premise of his contention seeking disclosure of the source codes is that a criminal defendant need not make a prima facie showing of the relevance and necessity of the trade secret before disclosure occurs. Rather, upon a defense request for material that qualifies as a trade secret, the holder of the trade secret privilege cannot object on the ground that no showing of relevance and necessity has been made. To the contrary, the privilege holder's only remedies, even for material as to which there is no relevance and necessity, are to seek a protective order limiting the terms of disclosure (but not precluding disclosure) under Evidence Code section 1061, and closing the proceedings at which the trade secret might be disclosed under Evidence Code section 1062.

We decline to read Evidence Code sections 1061 and 1062 in such a manner. In short, it makes no sense to require the holder of a trade secret privilege to submit to disclosure of the trade secret, even subject to a protective order and the closing of certain proceedings, without a showing that the trade secret is relevant and necessary to the defense. (See *People v. Superior Court* (*Barrett*) (2000) 80 Cal.App.4th 1305, 1318 ["A criminal defendant has a right to discovery by a subpoena duces tecum of third party records on a showing of good cause -- that is, specific facts justifying discovery."].) We thus conclude that the test for trade

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"(e) Whenever the court closes a portion of a criminal proceeding pursuant to this section, a transcript of that closed proceeding shall be made available to the public as soon as practicable. The court shall redact any information qualifying as a trade secret before making that transcript available.

"(f) The court, subject to Section 867 of the Penal Code, may allow witnesses who are bound by a protective order entered in the criminal proceeding protecting trade secrets, pursuant to Section 1061, to remain within the courtroom during the closed portion of the proceeding."

secret disclosure adopted in *Bridgestone* -- a prima facie, particularized showing that the source code is relevant and necessary to the defense -- is required for Chubbs to require disclosure of the source codes.

The trial court here correctly began with a determination under Evidence Code section 1060 that the source code is a trade secret and then moved to the issue of a protective order pursuant to Evidence Code section 1061. However, because we find that Chubbs did not meet his prima facie burden for disclosure, we conclude that the People should not have been compelled to produce the source code, whether or not subject to a protective order.

II. *Chubbs' Evidence Regarding Necessity of Source Code*

Chubbs submitted declarations from defense counsel and Jamieson to support his contention that the source code is essential to his defense.[11] Defense counsel relied on the fact that the DNA evidence was the only evidence connecting Chubbs to the victim.

Defense counsel further declared that without the source code, "there is no way for my expert to determine what assumptions, among other things, have been made and if they are appropriate in this particular case." In her application for the subpoena duces tecum, she declared that her DNA experts, another forensic DNA consultant (who has helped develop a program similar to TrueAllele with source codes open for public review), and unidentified attorneys "who focus on DNA

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11 Chubbs attached to his return a declaration from Dr. Travis Doom regarding the necessity of the source codes. We decline to consider the declaration because it was not submitted to the trial court. (See *Pomona Valley Hospital Medical Center v. Superior Court* (2013) 213 Cal.App.4th 828, 835, fn. 5 ["Writ review does not provide for consideration of evidence not before respondent court at the time of its ruling."].)

evidence regarding TrueAllele" advised her to request the source codes and pseudo source codes.

In Jamieson's declaration, he claimed ten years of forensic experience, as well as familiarity with TrueAllele's "claimed methodology and use" and "experience" in court with TrueAllele. He opined that "access to this code is the only satisfactory and professionally recommended way to fully consider the validity of the TrueAllele analysis" in this case. He claimed that others who have developed computer-assisted DNA comparison software "do not hide their source codes" and instead make them freely available, which allows others to fully review and verify the reliability of the method and results in any given case.

In considering whether Chubbs has made a prima facie showing of the necessity of the source code to his defense, we consider not only Chubbs' evidence but also Perlin's declaration, which was submitted in support of the People's motion to quash the subpoena duces tecum. (See *Bridgestone*, *supra*, 7 Cal.App.4th at p. 1395 ["while the burden of making a prima facie showing of the particularized need for a trade secret is on the party seeking discovery, the trial court need not ignore evidence presented by the opposing party on the question whether the information sought is a trade secret"].)

Perlin explained that TrueAllele is useful when uncertainty in DNA analysis arises, such as when two or more people contribute to the evidence, and it decreases uncertainty by comparing information to a suspect. TrueAllele is "Cybergenetics' computer implementation of [a] two-step DNA identification inference approach." This process involves, first, "objectively inferring genotypes from evidence data, accounting for allele pair uncertainty using probability," and "subsequently matching genotypes, comparing evidence with a suspect relative to a population, to express the strength of association using probability."

Perlin declared that TrueAllele is widely accepted, having been used in approximately 200 criminal cases in courts in California, Pennsylvania, and Virginia, and it has been subjected to numerous validation studies, five of which were published in peer-reviewed scientific journals.

Perlin explained that software source code is the programming language used to write a computer program. The source code "details step-by-step human-readable instructions that describe to the computer and programmers how the program operates," and is "translated into computer-readable 'executable' software." He stated that TrueAllele has about 170,000 lines of computer source code and opined that reading through the source code would not yield meaningful information.

As to the proprietary nature of the source code, Perlin explained that others "can easily copy a computer program if they have its source code," which "contains the software design, engineering know-how, and the algorithmic implementation of the entire computer program." Cybergenetics has invested millions of dollars over 20 years to develop TrueAllele, which it offers to crime labs for a base license fee of $60,000.

Perlin differentiated TrueAllele from the open source DNA analysis software programs referenced in the declarations of defense counsel and Jamieson, stating that open source programs "typically are not validated prior to release, because the process of perfecting software is costly." In addition, open source forensic programs "tend to be relatively short programs consisting of several hundreds of lines of code," in contrast to the 170,000 lines of code in TrueAllele.

Cybergenetics accordingly has never disclosed the source code to anyone outside the company and does not distribute it to businesses or government agencies that license the software. Cybergenetics does, however, disclose

TrueAllele's methodology and its "underlying mathematical model" to enable others to understand its genotype modeling mechanism. The company "provides opposing experts the opportunity to review the TrueAllele process, examine results, and ask questions."

Cybergenetics keeps the source code secret because of the "highly competitive commercial environment" in which it operates. Perlin declared that Cybergenetics' competitors are interested in replicating TrueAllele and that disclosure of the source code would enable its competitors to copy the product, causing the company irreparable harm. Perlin believed that source code is not revealed for other commercial forensic DNA software because the source code is not needed to assess the software programs' reliability.

Jamieson's general statement that a criminal defendant cannot "receive a diligent and fair verification of a DNA testing or analysis method" without the source codes does not address Perlin's explanations of what the source code actually is and why it is not needed to test the methodology or reliability of TrueAllele's analysis. Jamieson also generally states that access to the source code is the only way to consider the validity of the TrueAllele analysis in Chubbs' case, but he does not explain how access to the source code would allow him to test the reliability of TrueAllele's analysis. (See *Bridgestone*, *supra*, 7 Cal.App.4th at p. 1396 ["nowhere did [the real parties' expert] describe with any precision how or why the [trade secret] formulas were a predicate to his ability to reach conclusions in the case"].)

Similarly, defense counsel generally states in her declaration that others have told her she needs to request the source codes and that there is "no way [she] can properly prepare to defend against the TrueAllele results without the source codes and pseudo source codes." However, these general declarations do not address

why the source code is needed to review the reliability of the TrueAllele analysis, how the source code would be used to review the TrueAllele results, or what could be revealed by the source codes that would be useful to Chubbs' defense. Indeed, her declarations regarding TrueAllele's methodology, inferences, and reliance on the likelihood ratio rather than the random match probability illustrate her understanding of TrueAllele and thus undercut her argument that the source codes are necessary to understanding TrueAllele. This is particularly true in light of the fact that defense counsel received the patent documents regarding TrueAllele, numerous published articles regarding TrueAllele, and TrueAllele operating manuals. Further supporting the position that the source code is not necessary to an understanding of TrueAllele is Perlin's statement in his declaration that Cybergenetics discloses to opposing experts TrueAllele's methodology, how it applies its method to the data, and how the software works. The supplemental report prepared by Cybergenetics also explained the assumptions made by TrueAllele in its analysis. The vague statements by defense counsel and Jamieson do not describe in any way how the source code would have any bearing on the reliability of the analysis.

In his declaration, Perlin cited *Commonwealth v. Foley* (Pa. Super. 2012) 38 A.3d 882, in which the Superior Court of Pennsylvania held that the trial court did not abuse its discretion in admitting Perlin's DNA-related testimony. (*Id.* at p. 890.) Although this out-of-state case does not carry precedential weight, we agree with its conclusion that access to TrueAllele's source code is not necessary to judge the software's reliability. Similar to Chubbs' case, Perlin's estimate of the probability of a DNA match to the defendant in *Foley* was much higher (1 in 189 billion) than the estimates of the other scientific experts (1 in 13,000 and 1 in 23 million). (See *id.* at p. 887.) As pertinent here, the Pennsylvania court rejected the

defendant's argument that Perlin's testimony should have been excluded, reasoning that "scientists can validate the reliability of a computerized process even if the 'source code' underlying that process is not available to the public. TrueAllele is proprietary software; it would not be possible to market TrueAllele if it were available for free. [Citation.]" (*Id.* at p. 889.) The court further reasoned that TrueAllele "has been tested and validated in peer-reviewed studies," citing several papers that "were published in peer-reviewed journals" and thus "reviewed by other scholars in the field." (*Id.* at pp. 889-890.)

"[I]t is not enough that a trade secret might be useful to real parties." (*Bridgestone*, *supra*, 7 Cal.App.4th at p. 1395.) Instead, "the party seeking discovery must make a prima facie, particularized showing that the information sought is relevant and necessary to the proof of, or defense against, a material element of one or more causes of action presented in the case, and that it is reasonable to conclude that the information sought is essential to a fair resolution of the lawsuit." (*Id.* at p. 1393.) Chubbs has received extensive information regarding TrueAllele's methodology and underlying assumptions, but he has not demonstrated how TrueAllele's source code is necessary to his ability to test the reliability of its results. We therefore conclude that Chubbs has not made a prima facie showing of the particularized need for TrueAllele's source code.

III. *Right to Confront Witnesses*

The trial court relied on Chubbs' constitutional right to confrontation to conclude that the People were required to produce the source code. However, our state supreme court has stated, "invocation of the confrontation or compulsory process clauses in a claim involving pretrial discovery 'is on weak footing' because it is unclear whether or to what extent those constitutional guarantees

grant pretrial discovery rights to a defendant. [Citations.]" (*People v. Clark* (2011) 52 Cal.4th 856, 982-983; see also *People v. Hammon* (1997) 15 Cal.4th 1117, 1126 [examining United States Supreme Court precedent and concluding, "it is not at all clear 'whether or to what extent the confrontation or compulsory process clauses of the Sixth Amendment grant pretrial discovery rights to the accused'"] (*Hammon*).)

"In [*Hammon*], the Supreme Court held the trial court properly quashed a subpoena duces tecum the defendant served on psychotherapists treating the alleged victim without first conducting an in camera review of the material. '[R]eject[ing the] defendant's claim that pretrial access to such information was necessary to vindicate his federal constitutional rights to confront and cross-examine the complaining witness at trial or to receive a fair trial' [citation], *Hammon* held 'the trial court was not required, at the pretrial stage of the proceedings, to review or grant discovery of privileged information in the hands of third party psychotherapy providers' [citation]." (*People v. Petronella* (2013) 218 Cal.App.4th 945, 958 (*Petronella*).)

Hammon reasoned that United States Supreme Court precedent addressing a criminal defendant's right under the confrontation clause to information protected by state-created evidentiary privileges applied to a defendant's trial rights, not pretrial rights. (*Hammon*, *supra*, 15 Cal.4th at pp. 1123-1127.) The court further reasoned that, "[w]hen a defendant proposes to impeach a critical prosecution witness with questions that call for privileged information, the trial court may be called upon . . . to balance the defendant's need for cross-examination and the state policies the privilege is intended to serve. [Citation.] Before trial, the court typically will not have sufficient information to conduct this inquiry; hence, if pretrial disclosure is permitted, a serious risk arises that privileged material will be

disclosed unnecessarily." (*Id.* at p. 1127.) The court thus "decline[d] to extend the defendant's Sixth Amendment rights of confrontation and cross-examination to authorize pretrial disclosure of privileged information." (*Id.* at p. 1128.)

Similarly, *Petronella* concluded that the trial court's pretrial ruling upholding a privilege claim against the defendant's subpoena did not violate the defendant's constitutional rights to confrontation and due process. (*Petronella*, *supra*, 218 Cal.App.4th at pp. 958-959.) Pursuant to *Hammon* and *Petronella*, we conclude that Chubbs' right to confrontation does not apply to pretrial discovery of the source code, which is privileged information.

Chubbs relies on the concurring and dissenting opinions in *Pennsylvania v. Ritchie* (1987) 480 U.S. 39 (*Ritchie*) to argue that the confrontation clause applies to pretrial discovery. However, *Hammon* specifically addressed *Ritchie* in concluding that the Sixth Amendment right to confrontation did not confer a right to discover privileged information before trial. (*Hammon*, *supra*, 15 Cal.4th at pp. 1125-1127.) We therefore conclude that the trial court abused its discretion in relying on the confrontation clause to order disclosure of the TrueAllele source codes.

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DISPOSITION

Let a peremptory writ of mandate issue directing respondent court to vacate its order compelling disclosure of the source code, and to issue a new order denying the motion to compel discovery.

NOT TO BE PUBLISHED IN THE OFFICIAL REPORTS

WILLHITE, J.

We concur:

EPSTEIN, P. J.

MANELLA, J.

EXHIBIT 16

At a Term of the Supreme Court of the State of New York held for the County of Schenectady, New York at Chambers in the Village of Cooperstown, New York on the 13 day of March, 2015

PRESENT: HON. MICHAEL V. COCCOMA
SUPREME COURT JUSTICE

STATE OF NEW YORK
SUPREME COURT: COUNTY OF SCHENECTADY

THE PEOPLE OF THE STATE OF NEW YORK

-against-

JOHN WAKEFIELD

Defendant

DECISION AND ORDER

Indictment No. A-812-29

Notwithstanding the fact that the Court has already ruled on the Defendant's right to the Cybergenetics TrueAllele Casework's source code (see Decision and Order dated February 9, 2015 at pages 6 - 7), and ignoring the timeliness issue, the Court will address this Motion on the merits.

The Defendant argues that the TrueAllele Casework System is an expert system which interpreted DNA data in this case, drew inferences from it, and reached the conclusions directly connecting Mr. Wakefield to the crime with which he has been charged. To begin with, such an argument ignores the human element, to wit: the analyst. Secondly, the DNA results from Cybergenetics TrueAllele Casework is not a hearsay statement by an individual against the

Defendant - it is a scientific report generated from the source code. Thirdly, and more importantly, the Defendant has not forfeited his right to confrontation since he will have an opportunity to cross-examine not only the analyst, but the scientist who developed the software.

Simply put, the Defendant's Crawford argument is misplaced. The source code is not a witnesses, it is not testimonial in nature, and it is not "a surrogate for accusatory in-court testimony." It is only the software that drives a computer program that analyzes DNA with the input and assistance of an analyst. And the Cybergenetics TrueAllele Casework report does not accuse anyone, it simply computes a match likelihood ratio using a probabilistic model.

Accordingly, the Motion to allow the Defendant's expert access to the Cybergenetics TrueAllele Casework source code is DENIED once again.

THIS SHALL CONSTITUTE THE DECISION AND ORDER OF THE COURT.

Dated: March 13, 2015
at Cooperstown, New York

ENTER

Hon. Michael V. Coccoma
Supreme Court Justice

To: John Wakefield
Frederick Rench, Esq.
Catherine Bonventre, Esq.
Peter H. Willis, ADA, Schenectady County District Attorney's Office
Clerk of the Court

The documents upon which this Decision and Order is based have been filed in the Office of the Schenectady County Clerk:

1. Memorandum of Law dated March 10, 2015
2. Letter from Peter H. Willis, Assistant District Attorney, dated March 13, 2015 showing copy to Defendant.

EXHIBIT 17

IN THE COURT OF COMMON PLEAS OF ALLEGHENY COUNTY, PENNSYLVANIA
CRIMINAL DIVISION

COMMONWEALTH OF PENNSYLVANIA	)	
	)	
v.	)	**CC 201307777**
	)	
MICHAEL ROBINSON,	)	
	)	
Defendant	)	

MEMORANDUM ORDER

AND NOW, to-wit, this 4th day of February, 2016, this Court hereby DENIES Defendant's "Application Pursuant to Title 42 Pa.C.S.A. Section 702(B), Interlocutory Orders, for Amendment to Include Certification of the Interlocutory Discovery Order Issued on December 7, 2015." This Court denied Defendant's discovery request for the "source code" for Cybergenetics TrueAllele Casework System, which was used to test a bandana recovered from the crime scene which the Commonwealth alleges belongs to Defendant. This source code is the intellectual property of Cybergenetics.

Pa. R. Crim. P. 573 states that a trial court may permit discovery of items which are material, reasonable and in the interests of justice, and Defendant asserts that his request for the source code has met this criteria. However, "[e]vidence is material only if there is a reasonable probability that, had the evidence been disclosed to the defense, the result of the proceeding would have been different. A 'reasonable probability' is a probability sufficient to undermine confidence in the outcome." *Pennsylvania v. Ritchie*, 480 U.S. 39, 57 (1987). Since materiality requires that the material sought must be outcome-determinative (*See also Commonwealth v. Tharp*, 101 A.3d 736, 748 (Pa. 2014)), Defendant must establish that production of the source

code is a linchpin to undermining the Commonwealth's case as it pertains to the DNA evidence on the bandana.

In support of its assertion, Defendant alleges that TrueAllele's reliability cannot be evaluated without the source code. The Pennsylvania Superior Court, in *Commonwealth v. Foley*, 38 A.3d 882 (Pa. Super. 2012) (*en banc*), disagreed. The *Foley* court discussed whether TrueAllele testing was admissible pursuant to *Frye v. United States*, 293 F. 1013 (D.C. Cir. 1923) and in so doing found that TrueAllele was not "novel" science. *Foley* addressed the issue of assessing the reliability of TrueAllele without the production of the source codes and determined that scientists could validate the reliability of TrueAllele without the source code. *Id.* at 889-90. In addition, the *Foley* court noted that the trial court had "[found] Dr. Perlin's methodology [to be] a refined application of the "product rule," a method for calculating probabilities that is used in forensic DNA analysis." *Foley*, 38 A.3d at 888. The Superior Court noted that evidence based on the product rule previously has been deemed admissible under *Frye*. *Id.*, citing *Commonwealth v. Blasioli*, 713 A.2d 117, 1118 (Pa. 1998).

As the defense has argued that *Foley* is not controlling on the question of materiality of the source code, this Court held a two day hearing and considered expert testimony and argument. After considering the testimony, this Court determined that the source code is not material to the defendant's ability to pursue a defense.

Moreover, release of the source code would not be reasonable under Pa. R. Crim. Pro. 573 (A). Dr. Mark Perlin, founder of Cybergenetics, stated in his April 2015 Declaration that disclosure of the source code would cause irreparable harm to the company, as other companies would be able to copy the code and potentially put him out of business. (Commonwealth's Supplemental Answer to Motion for Discovery, Exhibit 1, "Declaration of Mark W. Perlin, April

2015" para. 54-55) An order requiring Cybergenetics to produce the source code would be unreasonable, as release would have the potential to cause great harm to Cybergenetics. Rather than comply, Dr. Perlin could decline to act as a Commonwealth expert, thereby seriously handicapping the Commonwealth's case.

42 Pa.C.S. § 702(b) states that if the trial court believes the interlocutory order "involves a controlling question of law as to which there is substantial ground for difference of opinion and that an immediate appeal from this order may materially advance the ultimate termination of the matter, it shall so state in such order." This Court is not of the opinion that the discoverability of the source code for Cybergenetics' TrueAllele Casework system involves a controlling issue of law to which a substantial ground for a difference of opinion exists. Defendant alleges that the Honorable Jeffrey A. Manning's ruling in the *State of California v. Martell Chubbs* creates a substantial ground for a difference of opinion. However, in that case J. Manning merely enforced a subpoena *duces tecum* ordering Dr. Perlin to appear in California with the documents subject to the subpoena but he left the ultimate disposition of the discovery request to the California court. Ultimately, the California Superior Court did not require Cybergenetics to produce the source code.[1] Further, J. Manning, in another pending matter involving a discovery request for the TrueAllele source code, declined[2] to read his ruling in *Chubbs* as controlling or contradictory and deferred to this Court for a ruling on the issue of the discoverability of source code. Similarly, the Honorable Edward J. Borkowski, without a hearing, quashed a subpoena *duces tecum* requesting production of the TrueAllele source code in another case pending in this this Court.[3]

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[1] 2015 WL 139069 (Unpublished Opinion)
[2] *Commonwealth v. Chelsea Arganda and Chester White*, CC# 2013-17748 and CC# 2013-17753.
[3] *Commonwealth v. Wade*, CC# 2014-04799.

Reviewing *Foley* and *Chubb*, as well as the pretrial proceedings of record in other matters pending before my colleagues in the Criminal division of the Court of Common Pleas of Allegheny County, and taking into consideration the briefs and arguments of the parties, this Court finds no reason to certify its December 7, 2015 Discovery Order for Interlocutory Appeal.

BY THE COURT:

Jill E. Rangos, J.
Honorable Jill E. Rangos

EXHIBIT 18

SUPERIOR COURT OF WASHINGTON FOR KING COUNTY

STATE OF WASHINGTON, Plaintiff, vs. EMANUEL FAIR, Defendant.	No. 10-1-09274-5 SEA FINDINGS OF FACT AND CONCLUSIONS OF LAW ON DEFENSE MOTION TO COMPEL CYBERGENETICS' TRUEALLELE CASEWORK SOURCE CODE

A hearing on the Defense Motion to Compel Cybergenetics' TrueAllele Casework Source Code was heard from October 31 to November 28, 2016. After considering the evidence submitted by the parties, to wit: the Defense Motion to Compel Cybergenetics' TrueAllele Casework Source Code, the State's Response to Defense Motion to Compel TrueAllele Source Code, the Defense Reply Regarding Motion to Compel TrueAllele Source Code, the exhibits attached to the pleadings, the testimony from witnesses including Jay Caponera, Nathan Adams, Dan Krane, Mark Perlin, David Balding, Kirk Lohmueller, and Brian Ferguson, the exhibits offered into evidence and hearing argument, the court makes the following findings of fact and conclusions of law:

A. FINDINGS OF FACT

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1. The Court heard testimony from Nathan Adams, a systems engineer at Forensic Bioinformatics in Dayton, Ohio. Bioinformatics is a DNA consulting company founded by Dr. Daniel Krane. Mr. Adams has a B.S. in Computer Science and is working towards obtaining his M.S. in computer science.

2. Mr. Adams testified that source code is the human language that a computer can understand and translate to machine language in order to execute its operations. TrueAllele software contains 170,000 lines of code.

3. Mr. Adams testified that he had reviewed the source code of another probabilistic genotyping system (PGS) called STRmix under a protective order and that because of that order, he was unable to share any specific findings from his source code review.

4. Mr. Adams testified that his review of STRmix's source code occurred with numerous precautions, in addition to the protective order, to insure that the code would not be stolen. Mr. Adams was not allowed to bring any photographic, recording, or USB devices into the room where the review occurred, the computer on which he reviewed the code was disconnected from the internet, and that he was monitored at all times by an armed guard.

5. Mr. Adams testified that in 30 hours he was able to identify potential issues in STRmix's source code that negatively affected the functioning of the software that could not have been learned from any other source. However, due to the protective order, Mr. Adams could not disclose what those potential issues were.

6. Mr. Adams testified that there are three levels of source code review. First, a dedicated software firm could be hired to review the code for possible errors. This would cost hundreds of thousands of dollars. A mid-range review involving a 200 hour review of the code would cost approximately $40,000 at $200 per hour. This would take several months. Lastly, a brief

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20 hour review could provide insight into the general practices and standards of the code but would not allow a thorough investigation of all the models of molecular behavior.

7. The Court heard testimony about a number of different PGS products. Many PGS are open source, meaning the source code of the software can be reviewed by the public. PGS including TrueAllele, STRmix, and FST are proprietary and do not publish their source code. STRmix and FST have disclosed their source codes pursuant to court order. TrueAllele has not been ordered to disclose its source code[1].

8. Brian Ferguson is a lawyer with over twenty years' experience in intellectual property litigation. Mr. Ferguson is the co-chair of the Patent Litigation department at the law firm of Weil, Gotshal, and Manges, LLP, in Washington, D.C. Mr. Ferguson's work focuses on patent infringement cases involving disputes between companies regarding whether or not a particular patent has been violated.

9. Mr. Ferguson testified that source code is disclosed in intellectual property litigation because that is the only way for the particular functionality of the product to be assessed. If a dispute arises between smartphone companies over whether a particular function of a smartphone or tablet has been copied by a rival company, there is a need to determine how the software was programmed. That can only be done by reviewing the source code.

10. Mr. Ferguson testified that in intellectual property litigation, the parties retain a software engineering expert to review the source code with guidance from a subject matter expert. The subject matter expert will review the source code with the attorneys to identify the particular functionality in the patent that is key to determining whether or not an infringement has occurred. Then the source code expert will focus on reviewing that functionality in the source code.

[1] A California trial court did order Cybergenetics to disclose its source code but this order was later overturned on appeal. *People v. Superior Court (Chubbs)*, 2015 WL Reporter 139069.

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11. The defense made an oral offer of proof to the Court that experts like Dr. Krane, Mr. Adams, Dr. Lohmueller, or Dr. Balding could be used as subject matter experts should the Court order TrueAllele's source code disclosed. Additionally, the defense made an oral offer of proof that it had contacted a software engineer expert who was qualified and available to review the source code itself.

12. Dr. Balding testified that he not understand how TrueAllele performs some of its functions, including how it models drop out and drop in and that no publication or document describes how TrueAllele accounts for drop-out. Dr. Balding further testified that he wrote the original source code for LikeLTD but the current version was written by software engineers and he hasn't reviewed it.

13. Dr. Lohmueller testified that TrueAllele's source code would be helpful in understanding how TrueAllele behaves when it is modelling samples where there is the possibility of drop-out. Dr. Loehmueller further testified that scientists can test data without the source code. The source code is only one piece of the validation process. In fact, he has never looked at the source code for his own PGS, Lab Retriever.

14. Dr. Krane testified that first and foremost he is a Biology professor and has no formal degrees in math or statistics. He testified that he could not review TrueAllele's source code entirely by himself. He would need at least a team of at least a dozen software engineers to do a comprehensive review although even a 40 hour review might reveal something important. He testified that the source code would be helpful to understand how the software deconvolutes mixtures; distinguishes signal from noise when looking at peaks as low as 10/0 RFU; and identifies peaks and peak heights, which TrueAllele does using a method different than any other PGS.

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15. Dr. Mark Perlin, founder of Cybergenetics, testified that his company has invested millions of dollars over the last two decades to develop the TrueAllele software. The technology is patented but the source code has never been revealed in any patent. Cybergenetics considers the TrueAllele source code to be a trade secret. Dr. Perlin testified that disclosure of the TrueAllele source code would allow competitors to copy the software and cause irreparable financial harm to his company.

16. Dr. Perlin further testified that disclosure of the source code is not necessary to validate the reliability of the program.

17. Jay Caponera, a forensic scientist with the New York State Police, testified that the source code is not necessary to determine the reliability of TrueAllele because the code is not used in validation. Reliability of software is determined by use of the validation metrics of sensitivity, specificity, accuracy and reproducibility. He testified that he validated TrueAllele in 2011 without access to the source code.

18. John Donahue is employed as the DNA Technical Leader at the Beaufort County Sheriff's Office Forensic Services Laboratory in Beaufort, South Carolina. In his Declaration, he testified that his lab has used TrueAllele for three years. They purchased the software in 2013 and spent two years performing validation studies before implanting it into casework in January 2016. He testified that the source code was not necessary to determine the reliability of TrueAllele because in their validation studies they tested TrueAllele against known samples and known results and obtained the expected results.

19. Thomas Hebert is employed as the DNA Technical Leader for the Baltimore City Police Department. In his Declaration, Mr. Hebert testified that his lab has used TrueAllele for casework since October 2015. In his opinion, the source code is not necessary to determine the

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reliability of software programs for forensic used. A proper validation requires testing samples with known results. These results can then be compared to results generated by the program. A wide variety of samples should be used to simulate real casework type samples to show the limits of the software.

20. Kevin Miller is employed as the Forensic Scientist Leader at Hamilton Robotics. In his Declaration, he testified that he assisted the Kern County Regional Crime Laboratory in California in purchasing and validating TrueAllele for casework in 2014. He testified that DNA analysts are not required to have the computer science or engineering backgrounds required to review source code. Forensic analysts rely on instrumentation to perform a wide array of mathematical calculations without requiring the analysts to check the calculations or know the source code for the procedures.

21. Joanne Sgueglia was previously employed at the Massachusetts State Police Crime Laboratory where TrueAllele was validated in 2011. In her Declaration, Ms. Sgueglia testified that she has been involved in forensic DNA research and development/validation efforts for over 28 years. She testified that knowledge of the source code is not needed to validate TrueAllele. In the field of forensics, labs evaluate and validate many systems by testing without specific knowledge of the underlying mechanisms, programming, algorithms or chemistry.

22. Dr. Gary Shutler is employed as the DNA Technical Leader for the Washington State Patrol Crime Laboratory (WSPCL). In his Declaration, Dr. Shutler testified that the WSPCL does not currently have the funds to do probabilistic genotyping in their laboratory so it contracts with Cybergenetics for interpretation of complex DNA mixtures. Dr. Shutler testified that the WSPCL uses a variety of software technologies in their lab (such as GeneMapper and PopStats) and has never found it necessary to review the source code to establish validation.

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23. Dr. Susan Greenspoon is employed as a Molecular Biologist at the Virginia Department of Forensic Science. In her April 4, 2016, letter, she wrote that the internal validation study performed in her laboratory assessed TrueAllele's accuracy, reproducibility, sensitivity (ability to detect minor contributors) and specificity (ability to eliminate non-contributors) without the need for the source code.

24. The Scientific Working Group on DNA Analysis Methods (SWGDAM) is a group of approximately 50 scientists representing federal, state and local forensic DNA laboratories in the United States and Canada. They meet twice a year and issue documents to provide direction and guidance for the scientific community. The 2015 SWGDAM Guidelines for the Validation of Probabilistic Genotyping Systems do not require or even mention the need for a computer source code for validation.

25. 34 validation studies of TrueAllele have been published. Seven have been published in peer-reviewed journals. Ex 44. None of the validation studies included a review of the source code.

26. Cybergenetics provided the defense with a case report and case packet containing 4 GB of information detailing the testing done in this case. Additionally, Cybergenetics provides defense experts with a 96-day license to use TrueAllele in a read-only viewer and the ability to test their own mixtures using their own data on the TrueAllele on the Cloud at no charge.

B. CONCLUSIONS OF LAW

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1. The discovery which the defense seeks is not in the State's possession. Dr. Perlin advised the State that Cybergenetics considers the TrueAllele source code to be a trade secret and would not be providing it. CrR 4.7(d).

2. The Court can order the disclosure of materials outside of the required disclosures under CrR 4.7(a), (c), (d), if the information sought is material and the discovery request is reasonable. CrR 4.7(e)(1), *State v. Norby*, 122 Wn.2d 258 (1993).

3. The court can condition or deny disclosure if it finds that there is a substantial risk of harm or unnecessary annoyance resulting from such disclosure which outweighs the usefulness of any disclosure to the defendant. CrR 4.7(e)(2).

4. Materiality requires that the defendant "make a particularized factual showing that information useful to the defense is likely to be found in the records." *State v. Diemel*, 81 Wn.App. 464, 469 (1996).

5. The Defense has not articulated with particularity what material information, if any, could be found by reviewing the source code. As several experts who work in the field of forensic DNA testing have testified, an examination of the source code is not necessary in order to determine the reliability of TrueAllele and validate it for casework.

6. This is not a situation where production of the source code is necessary so that a particular functionality of the software can be examined to see if a patent infringement has occurred.

7. TrueAllele has been validated for use in casework by laboratories in California, Louisiana, Maryland, New York, Ohio, Pennsylvania, South Carolina, Virginia, Northern Ireland and Australia without having access to the source code.

8. The Defense has failed to meet its burden to show that disclosure of the source code is material and reasonable. Based upon the factual findings set forth above, this Court is not

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persuaded that a review of the source code is necessary in order to determine whether TrueAllele is reliable. The defense demand for the source code is not material or reasonable because the testimony in this case from both state and defense experts establishes that scientists can confirm the reliability of Trueallele without access to the source code. This testimony is consistent with the holding of other courts that have addressed this same issue. *State v. Wakefield*, 47 Misc. 3d 850, 854, 9 N.Y.S.3d 540, 543 (N.Y. Sup. Ct. 2015) ("scientists can, and have, validated the reliability of Cybergenetics TrueAllele Casework even though the source code underlying the process is not available to the public."); *Com. v. Foley*, 38 A.3d 882, 889 (Penn. Sup. Ct. 2012) ("scientists can validate the reliability of a computerized process even if the 'source code' underlying that process is not available to the public.").

9. Further, the usefulness of disclosing the source code is outweighed by a substantial risk of financial harm to Cybergenetics. Scientists can confirm the reliability of Trueallele without access to the source code. Dr. Perlin and Cybergenetics have a legitimate interest in keeping the source code, a trade secret, confidential.

C. ORDER

For the reasons stated above, the defendant's motion to compel the disclosure of TrueAllele's source code is DENIED.

Signed this _____ day of January, 2017.

e-filed
THE HONORABLE MARIANE SPEARMAN

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King County Superior Court
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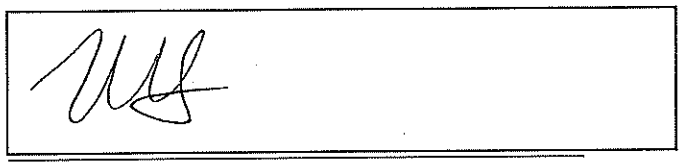
Case Title: STATE OF WASHINGTON VS FAIR, EMANUEL DEMELVIN AKA
Document Title: ORDER

Signed by: Mariane Spearman
Date: 1/12/2017 2:06:07 PM



Judge/Commissioner: Mariane Spearman

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EXHIBIT 19

Certificate of Completion

Lisa Schiermeier-Wood

Completed the TrueAllele® Casework course

Solving Cases: Levels 1 & 2

December 2012

Richmond, VA

Cybergenetics

Mark W. Perlin, PhD, MD, PhD
Chief Scientific Officer

EXHIBIT 20

Commonwealth of Virginia v. Clark Watson
Cybergenetics response to Defendant's Motion for a Certificate to compel the production of documents

September 17, 2019

Materials

- Cybergenetics TrueAllele DVD (provided with response)

Response

b. *All materials generated by [laboratory] when performing validation studies concerning the TrueAllele® system, including, but not limited to:*
   i. *All records and electronic data used as "input" to the TrueAllele system and the software parameters used to analyze this data.*

      Cybergenetics' electronic data for validation is provided on the TrueAllele DVD in the 2-Validation > 2-Data folder. Software parameters are described in the validation paper or report provided on the TrueAllele DVD in the 2-Validation > 1-Studies folder.

   ii. *All records and electronic data generated by the TrueAllele system and/or laboratory personnel during the course of the study.*

      No relevant materials.

   iii. *Any analyses of (3)a.i and 3)a.ii above), including bench notes, measurements, statistics, memos, summaries, conclusions, tables, graphics, and any resulting publications, presentations, and reports.*

      Validation papers and reports are provided on the TrueAllele DVD in the 2-Validation > 1-Studies folder.

   iv. *All communications relating to the design and results of the study, both within and external to the laboratory.*

      No relevant materials.

v. *All records of unexpected results, including false positives (false inclusions), false negatives (false exclusions), and the conditions under which the unexpected results were generated.*

False positives and negatives are described in the validation write-up for the study provided on the TrueAllele DVD in the 2-Validation > 1-Studies folder.

vi. *All records of software glitches, crashes, bugs, or errors encountered during the study.*

The software is described in the validation paper or report provided on the TrueAllele DVD in the 2-Validation > 1-Studies folder.

vii. *Software version numbers of the components of the TrueAllele® system used for the study.*

Software version numbers appear in the validation paper or report provided on the TrueAllele DVD in the 2-Validation > 1-Studies folder.

c. *All materials described in (3)a) that were produced by any other laboratory or entity concerning a validation study cited by [laboratory] as describing the capabilities and/or justifying the use of the TrueAllele® software system for casework.*

Validation reports are provided on the TrueAllele DVD in the 2-Validation > 1-Studies folder. Other materials are not available to Cybergenetics.

d. *Copies of all materials used for TrueAllele® training received or provided by [laboratory], including handouts, sample tests, presentations, and videos.*

Training videos are provided on the TrueAllele Case DVD in the 3-Tutorials folder. TrueAllele course syllabi, with URL links to materials, are provided on the TrueAllele DVD in the 5-Miscellaneous > 1-Training folder.

e. *Proficiency testing materials used by [laboratory], including materials used for testing, all materials submitted by a test-taker, and results of all tests taken.*

No relevant materials.

f. *Source code for the version(s) of the TrueAllele® system used in the instant case as well as the versions that apply to (3)a, 3)b, 3)c, and 3)d above), including all software dependencies such as third-party code libraries, toolboxes, plug-ins, and frameworks.*

An invitation to review TrueAllele source code by defense experts is provided on the TrueAllele DVD in the 4-VUIer > 6-Source code folder.

g. *Software engineering and development materials describing the development, deployment, and maintenance of the version(s) of the TrueAllele® software system used in the instant case as well as the versions that apply to (3)a, 3)b, 3)c, and 3)d above) including the software engineering documents recommended by organizations such as the Institute of Electrical and Electronics Engineers (IEEE) or the International Organization for Standardization (ISO) such as:*
   i. *Software requirements specification documents.*
   ii. *Software architecture and algorithm design documents.*
   iii. *Software implementation documents.*
   iv. *Software deployment documents.*
   v. *Software maintenance documents.*

No relevant materials.

h. *All documents relating to the above software engineering and development materials including, but not limited to:*
   i. *Modifications, revisions, or updates to original documents.*
   ii. *Software bug and issue tracking logs.*

No relevant materials.

iii. *Validation and testing documentation and reports for all levels of the software system hierarchy, including subroutines and software components, modules, and programs.*

Validation materials are provided on the TrueAllele DVD in the 2-Validation folder.

iv. *Requests for changes to the system made by any designer, developer, or user of the system.*

No relevant materials.

v. *Release, change, update, and upgrade descriptions and logs.*

Provided on the TrueAllele DVD in the 5-Miscellaneous > 2-VUIer updates folder.